INFLUENCE OF NITROGEN APPLICATION AND CROP RESIDUE ON DRIVERS OF MICROBIAL NITROUS OXIDE EMISSIONS IN CANOLA (*BRASSICA NAPUS* L.) PRODUCTION

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by

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ABSTRACT

Agricultural soils have been deemed as the major source of nitrous oxide (N_2O) emissions, which has been linked to global warming and has warming potential almost 300 times greater than carbon dioxide (CO_2). Canola (*Brassica napus* L.) is an important crop in western Canada. In Saskatchewan, canola production covers 34.8% of the seeded area, its cultivation depends heavily on nitrogen (N) fertilizer application and irrigation potential in Saskatchewan can boost canola production. Microbial communities play a critical role in soil N-cycling, and soil N_2O production is due to nitrifier and denitrifier activity, which is affected by agricultural management practices. To better understand the drivers of N_2O emissions that result from canola production, two studies were designed to (*i*) determine if different N fertilizer application rates and timing of application affects soil N-cycling genes, N-conversion enzymes activity and N_2O emissions in irrigated canola, and (*ii*) investigate why canola residue induce greater than expected N_2O emissions, using ^{13}C and ^{15}N labelled residues of wheat, pea, flax and canola to trace source of N_2O and CO_2 emissions.

The first study was a two-year field experiment designed to establish baseline information on the response of microbial N_2O production and consumption to different rates and timings of N application and management practices in irrigated canola. I used a combination of enzyme assays responsible for N conversions (urease and arylamidase), denitrification and nitrification assays and quantitative real-time PCR of key N functional genes to characterize changes in microbial potential for N_2O production and consumption. My findings showed that existing soil conditions prior to management events which were drier in 2015 than in 2016 affected the magnitude and possibly the source pathway of soil N_2O emission. Enzymatic response to N application was mainly affected by differences in soil moisture content. Ammonia-oxidizing bacteria (AOB) abundance increased with increasing rate of N application, while ammonia-oxidizing archaeal (AOA) remained stable, response within denitrifying community (*nirK*, *nirS* and *nosZ*) was only observable in 2016 with more evenly distributed precipitation as opposed to 2015. Increasing rate of N application increased N_2O emission whether N was applied at once or as split application.

To understand why greater than expected N_2O emissions are produced from canola residues, soil from the field study site was used in the second study. An incubation experiment using ^{15}N and

^{13}C labelled residues of canola, flax, pea and wheat to trace the source of N emitted as N_2O, and identify microbial drivers of residue decomposition and N_2O emissions, was set up. The magnitude of N_2O emission from residue amended soils was higher ($p < 0.05$) than non-amended soil and differed with residue type in the following order: canola > pea = wheat > flax > control. Residue addition significantly increased microbial abundance ($p < 0.001$), and induced higher denitrifier abundance ($p < 0.05$). Pearson correlation of dissolved organic carbon (DOC) and denitrification gene abundance was significant ($p < 0.05$) across all residue treatments. Both canola and pea residue addition resulted in an increased DOC ($p < 0.05$), but only canola residue resulted in decreased DON ($p < 0.05$) and NO_3^- ($p < 0.05$), showing an interplay between biologically available C and N that differed among residue types, affecting N_2O emissions. Analysis of $^{15}N_2O$ and $^{13}CO_2$ data revealed that N_2O production stimulated by canola residue addition was coming from the soil N pool and the stimulatory effect of canola residues on N_2O emissions is due to differences in microbial assimilation of residue C, causing a shift in microbial community structure based on residue types. Based on these results, existing soil moisture affects microbial N cycling gene abundance and functions which in-turn determined microbial N_2O emission following N and /or irrigation application. Residue addition also primed soil C availability, which led to increased microbial activity and rapid transformation of soil N pool, in addition to organic N from the residue.

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LIST OF ABBREVIATIONS

A	Actino
AAFC	Agriculture and Agri-Food Canada
AGR	Above ground residue
AMO	Ammonia monooxygenase
ANOVA	Analysis of variance
*amo*A	Gene encoding ammonia monooxygenase enzyme
AOA	Ammonia oxidizing archaea
AOB	Ammonia oxidizing bacteria
BGR	Below ground residue
BMPs	Best management practices
C	Carbon
CH_4	Methane
CO_2	Carbon dioxide
CSIDC	Canada-Saskatchewan Irrigation Diversification Centre
DEA	Denitrifying Enzyme Activity
DOC	Dissolved organic carbon
DON	Dissolved organic nitrogen
EF	Emission factor
F:B ratio	Fungi bacteria ratio
G-	Gram negative
G+	Gram positive
GHG	Greenhouse gas
GWC	Gravimetric water content
HAO	Hydroxylamine dehydrogenase
LSD	Least significant difference
MRPP	Multi-Response Permutation Procedure
N	Nitrogen
NH_3	Ammonia

NH_4^+-N	Ammonium-N
N_2O	Nitrous oxide
nirK	Gene encoding copper containing subunit of nitrite reductase
nirS	Gene encoding cytochrome cd1 containing subunit of nitrite reductase
NOB	Nitrite oxidizing bacteria
NO_2^-	Nitrite
NO_3^-	Nitrate
NO_3^-N	Nitrate-N
*nos*Z	Gene encoding catalytic subunit of nitrous oxide
O_2	Oxygen
PCoA	Principal Coordinate analysis
PLFA	Phospholipid fatty acid
PMMA	Poly methyl methacrylate
qPCR	Quantitative Real Time Polymerase Chain Reaction
RCBD	Randomized complete block design
SIP	Stable Isotope Probing
WFPS	Water filled pore space

1. INTRODUCTION

Nitrous oxide (N_2O) gas has consistently stimulated scientist interest, because of its contribution to radiative forcing by long-lived GHGs. It is a potent greenhouse gas (GHG). Nitrous oxide can persist in the atmosphere for more than 100 years, and has global warming potential 298 times greater than carbon dioxide (CO_2), contributing significantly to climate change (Hu et al., 2019; WMO, 2018; WMO, 2015; Signor and Cerri, 2013). About 6% of radiative forcing by GHGs is attributed to N_2O emission (Myhre et al., 2013). Various sources of N_2O emission include: oceans, soils, biomass burning, fertilizer use and various industrial processes. Emissions are driven by both natural processes (about 60%) and human activities (approximately 40%). Worldwide mean atmospheric N_2O concentration in 2017 reached 329.9±0.1 ppb, representing an increment of 122% over the 270 ppb pre-industrial N_2O emission value. The higher emissions are driven by the increase in fertilizer usage for crop production, and higher soil N_2O emission due to surplus deposition of atmospheric nitrogen (WMO, 2018)

According to Butterbach-Bahl et al. (2013), the dominant sources of N_2O are closely related to microbial production processes in soils, sediments and water bodies. Substantial amounts of GHGs are emitted from agricultural soils and are responsible for more than 50% of anthropogenic N_2O emissions (Carlson et al., 2017; IPCC, 2007). Agricultural emissions, owing to N fertilizer use and manure management, and emission from unmanaged/natural soils represent 56-70% of all global N_2O sources (Syakila and Kroeze, 2011). Crop residue amendments provide readily available C and N, as well as other nutrients to agricultural soils (Kumar and Goh, 1999). Microbial nitrification and denitrification play an important role in N_2O production in the soil, contributing approximately 70% of worldwide N_2O emission (Braker and Conrad, 2011; Syakila and Kroeze, 2011). These processes are affected by soil type, soil water content, oxygen (O_2) availability, nitrogen (N) fertilizer application, organic carbon (C) content, temperature, soil pH, and redox potential (Emerich et al., 2009; Stehfest and Bouwman, 2006; Rochester, 2003; Kumar and Goh, 1999). Increased production of leguminous crops is another agricultural contribution to N_2O emission through N being added by biological N_2-fixation (Lemke et al., 2018). However, lower N_2O emissions have been reported in legume-based rotation compared with the fertilized phase of crop rotation (Jeuffroy et al., 2013; Lemke et al., 2007).

With fixed land resources and expected increase in food demand due to increasing world population, farmers will need to increase crop production by enhancing productivity on existing lands through fertilizer and irrigation. It is estimated that almost two-thirds of future food needs must come from irrigated agriculture (FAO, 2017). Application of synthetic fertilizer as a nutrient source in agricultural systems has been rapidly increasing in recent years. Considerable amounts of residues remaining on the field after harvest can also provide readily available C and N (Kumar and Goh, 1999), which affects N_2O emission (Gao et al., 2016; Shan and Yan, 2013; Yao et al., 2013).

In 2018, over 8.9 million ha of canola was harvested in Canada, and Saskatchewan was responsible for over 50% of the harvested area (Statistics Canada, 2019). About 46% of total field crop area in Canada is located in Saskatchewan, with Saskatchewan irrigation potential, substantial portion of acreage can further be put into production through irrigation. To guarantee high crop yield, irrigated lands are more intensively managed compared with non-irrigated land requiring higher inputs of N fertilizer and greater pesticide, herbicide and fungicide use (Gianessi, 2013). Elevated N_2O emission after snowmelt, precipitation and fertilizer N application have been reported in previous studies (Lemke et al., 2018; Helgason et al., 2005). Soil mineral N accumulation in the post-harvest period under winter oilseed rape compared with winter cereal was considered responsible for increased N_2O emission from winter oilseed rape (Walter et al., 2015). Elevated soil inorganic N following canola, compared with cereal and legume crops have been reported (Lemke et al., 2018; Ryan et al., 2006; Kirkegaard et al., 1999).

Most field-based and laboratory experiments have focused on estimation, measurement and simulation of agricultural N_2O emissions (Rochette et al., 2018; Hu et al. 2015a; Rochette et al., 2008c). Understanding the microbial pathways of N_2O production in irrigated soils will enable development of more effective mitigation strategies to prevent N_2O emissions. Studying microbial decomposition of different crop residues using stable isotope tracers will help us to understand why certain crop residues cause higher than expected N_2O emission.

1.1 Research Objectives

The main objectives of this study were to (*i*) determine if different N fertilizer application rates and timing of application affects soil N cycling genes, N conversion enzymes activity and

N_2O emissions in irrigated canola, (*ii*) relate N fertilizer application rates and timing of N fertilizer application with abundance of N-cycling gene copies and N_2O emissions in irrigated canola, (*iii*) investigate why canola residues induce greater than expected N_2O emissions, and (*iv*) find relationship between active microbial communities and N_2O emissions. To address these objectives, two studies were designed to address the following hypotheses:

Study 1

1. Soil N-conversion enzyme activity and N-cycling gene abundance will increase with an increase in the application rate of fertilizer N.
2. Spring application of fertilizer N has a greater impact on soil N-conversion enzymes activity and N-cycling gene abundance than an in-season application of N.

Study 2

1. Nitrous oxide emissions following the addition of canola residues are greater than those following the addition of wheat residues
2. Nitrous oxide emissions respond to changes in soil N-cycling gene abundance.

1.2 Organization of the Thesis

The thesis is presented in manuscript format, in which each research study addresses each of the hypotheses. This thesis includes a detailed literature review (Chapter 2) followed by two research studies (Chapters 3 and 4), and synthesis of the two research chapters, conclusions and future work (Chapter 5). Chapter 3 establishes baseline information on microbial pathways of N_2O emissions in irrigated canola. Chapter 4 identifies microbial activities responsible for greater than expected residue-induced N_2O emission from canola residue. Each research study is written in such a way that it stands alone for submission to peer-reviewed journal.

2. LITERATURE REVIEW

2.1 Nitrous oxide

Global warming has been linked to changes in the concentration of greenhouse gases (GHG), mainly carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O). Anthropogenic activities have accelerated the rate of increase in the concentration of N_2O, CO_2 and CH_4 in the atmosphere (Mosier et al., 2006). Nitrous oxide has a global warming potential 298 times higher than CO_2 when considered over a 100-year timeline (Signor and Cerri, 2013). Agricultural systems are estimated to contribute almost 70% of anthropogenic N_2O emissions worldwide (Syakila and Kroeze, 2011; Farquharson and Baldock, 2008). In Canada, agricultural soils account for almost 50% of GHGs emissions from primary agriculture (Ellert and Janzen, 2008) and 70% of anthropogenic N_2O emissions as a result of N fertilizer application (Environment and Climate Change Canada, 2019). Nitrogen (N) fertilizer is essential for optimum crop yield, and the three prairie provinces of Alberta, Manitoba and Saskatchewan are responsible for 82% of all fertilizer N used in Canada (Gao et al., 2013), and this can greatly impact N_2O emission. Increased use of N fertilizer and organic manure to increase food production is expected to account for about 50% increase in N_2O emissions from agricultural soils (Us-Epa, 2006).

2.2 Nitrous oxide production in soil

Microbial processes of nitrification and denitrification mediate the production of N_2O in the soil (Gregorich et al., 2015). These microbially-driven N conversions and resulting N_2O depend on the aeration status of the soil, availability of inorganic N substrate, labile organic carbon (C), (Begum et al., 2014), temperature and water availability (Gao et al., 2013). Rainfall, irrigation and fertilization also drive N_2O emissions in soil (Gao et al., 2016). N_2O can also be produced in soil by non-biological processes of chemodenitrification and hydroxylamine oxidation, but the amount of N_2O produced in these ways is relatively insignificant (Signor and Cerri, 2013).

2.2.1 Nitrification

Nitrification is the process where ammonium-N (NH_4^+-N) is oxidized to nitrate-N (NO_3^-N) through the intermediate nitrite (NO_2^-). This microbially-mediated process involves two major

steps (Fig. 2.1) (Gregorich et al., 2015). The first step is the oxidation of ammonia (NH_3) to hydroxylamine (NH_2OH) and subsequent conversion to NO_2^-, which can only be performed by three groups of microorganisms, the ammonia-oxidizing bacteria (AOB) (Kowalchuk and Stephen, 2001), the ammonia-oxidizing archaea (AOA) (Bollmann et al., 2011; Könneke et al., 2005) and comammox bacteria (Stein, 2019; Pjevac et al., 2017; Daims et al., 2015; Van Kessel et al., 2015), using ammonia monooxygenase (AMO) enzymes (Alves et al., 2018; Pjevac et al., 2017). The second step is the subsequent conversion of NO_2^- to NO_3^- by nitrite oxidizing-bacteria (NOB) (Francis et al., 2007; Kowalchuk and Stephen, 2001). The direct oxidation of NH_2OH to NO_2^- has been challenged by the recent discovery by Caranto and Lancaster (2017), that the enzyme hydroxylamine dehydrogenase (HAO) of AOB oxidizes NH_2OH to NO rather than NO_2^- (Stein, 2019).

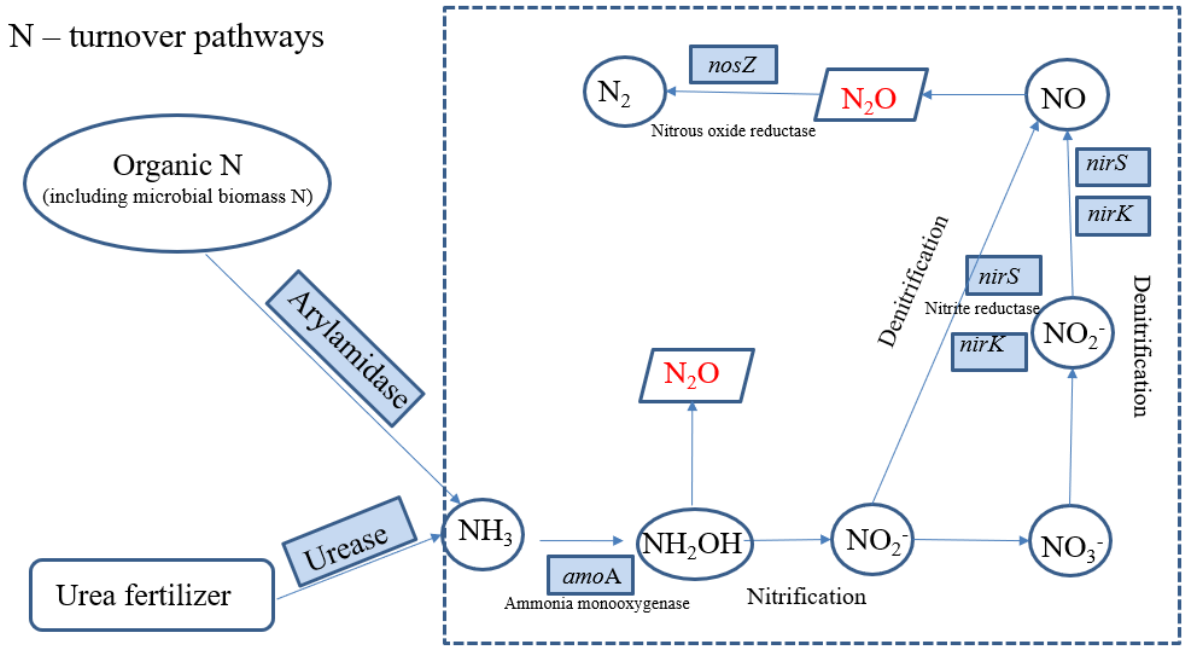

Fig. 2.1 Microbially mediated process of N_2O emission in soils (adapated from Figure 9.1 in Prosser, 2007).

Ammonia oxidation, a rate limiting step of nitrification, was presumed to be limited to AOB (O'Sullivan et al., 2011; Kowalchuk and Stephen, 2001; Bothe et al., 2000). However, the recent discovery of *amo*A and *nir*K genes in AOA strains negate the belief that nitrification in soils is

mainly performed by AOB (Li et al., 2013a). Nitrous oxide production by AOA enriched or purified from agricultural soils, and marine environments has also been demonstrated (Jung et al., 2014; Kim et al., 2012). The *amo*A gene is a key functional gene that encodes the alpha subunit of the ammonia monooxygenase enzymes, which are responsible for catalyzing the ammonia oxidation process. This *amo*A gene has been used by many researchers as a functional gene marker to detect changes in AOB and AOA populations (Pester et al., 2012; Junier et al., 2008; Rotthauwe et al., 1997). Contribution of AOA and AOB to nitrification is thought to be determined by the niche segregation for these ammonia-oxidizing microbes (He et al., 2018; Yin et al., 2018; Liu et al. 2014b). The niche segregation is influenced by factors such as ammonia concentration, pH and oxygen concentration (Bollmann et al., 2011; Gubry-Rangin et al., 2011; Verhamme et al., 2011; Molina et al., 2010). The abundance and diversity of AOA and AOB are affected by many factors such as NO_2^- and NO_3^- concentration, NH_4-N and soil conditions (Hayden and Beman, 2014; Liu et al., 2014b; Sun et al., 2014; French et al., 2012). The community structures of ammonia oxidizing organisms are greatly influenced by the amount and composition of organic matter, for example a high abundance of AOA has been documented in forest and long-term fallow lands with high labile organic carbon (Zeglin et al., 2011). Erguder et al. (2009), suggested that AOA plays a dominant role in soils with low pH and low N environments; this may be due to a soil pH effect on the community structure, abundance and activities of AOB and AOA.

2.2.2 **Denitrification**

Denitrification is a major microbial respiratory process that reduces oxidized mineral forms of N, NO_3^- and NO_2^-, to NO, N_2O and atmospheric N (N_2) in O_2 limiting conditions (Philippot et al., 2007). Though there is documented evidence of denitrification occurring in the presence of O_2 in the soil and sediments (Patureau et al., 2000). Intensive microbial respiration stimulated by C availability can create anaerobic microsites in which denitrification can take place (Abed et al., 2013).

Denitrification is a four-step reaction, each step of which occurs independently and can be performed by a diversity of bacteria, involving phylogenetically broad groups of microorganisms. The first step, conversion of NO_3^- to NO_2^- is catalyzed by *nar*G or *nap*A genes encoding NO_3^- reductase. The second step, conversion of NO_2^- to NO is catalyzed by *nir*K and *nir*S genes encoding two different types of nitrite reductase. The third step leading to the formation of N_2O (NO to

N_2O) is mediated by *cnor*B or *qnor*B genes encoding nitric oxide reductase, and the fourth step is the only known microbial process responsible for reduction of N_2O to N_2 by the *nos*Z gene encoding the nitrous oxide reductase (Hu et al., 2015a). Also, fungi could play a substantial role of denitrification in certain soils such as tropical arable peat, forest and grassland ecosystems (Thamdrup, 2012; Laughlin and Stevens, 2010; Yanai et al., 2007; Zumft, 1997), primarily producing N_2O, because fungi lack *nos*Z genes to further reduce N_2O to N_2 (Maeda et al., 2015; Baggs, 2011; Philippot et al., 2011). The denitrifying genes, *nir*K, *nir*S and *nos*Z have been well researched compared to other denitrifying genes such as *nar*G, *nap*A, *cnor*B and *qnor*B. The abundance, expression and metabolic activities of *nir*K, *nir*S and *nos*Z could be used as indicators for denitrification-derived N_2O changes in soils (Hu et al., 2015a; Morales et al., 2010). Two types of nitrite reductase enzymes (*nir*) catalyze the reduction of NO_2 to NO. They are the copper-containing nitrite reductase encoded by *nir*K and the cytochrome *cd1* nitrite reductase encoded by *nir*S (Zumft, 1997). The proportion of *nir*K and *nir*S to *nos*Z in a given soil can be related to N_2O emission capacity of the soil (Hu et al., 2015a). Almost one-third of bacterial strains containing *nir*K or *nir*S genes lack *nos*Z genes (Bakken et al., 2012; Philippot et al., 2011), the only known biotic sink for N_2O (Bru et al., 2011; Philippot et al., 2011).

Denitrification can be influenced by soil NO_3^--N content, type of tillage practices (Graham et al., 2013; Liu et al., 2007; Lemke et al., 2004), labile organic C (He et al., 2019; Yao et al., 2015), also subjective to water regime and mineral N levels in the soil, which can be regulated by irrigation and fertilization (Mosier et al., 2006). High input of mineral N fertilizer, after wetting event such as irrigation, rainfall and snowmelt can lead to significantly elevated emission of N_2O (Banerjee et al., 2016; Liu et al., 2011).

2.2.3 **Nitrifier-denitrification**

Nitrifier denitrification differs from coupled nitrification-denitrification in which nitrifiers provide product for denitrification (Wrage-Mönnig et al., 2018; Gregorich et al., 2015; Wrage et al., 2001). Nitrifier denitrification is a process carried out by autotrophic NH_3-oxidizing bacteria, under low soil O_2 concentration, by oxidizing NH_3 to NO_2^- and further reduction to N_2O and N_2 (Gregorich et al., 2015) (Fig. 2.1). Contrary to belief that AOA are not able to carry out nitrifier denitrification (Stieglmeier et al., 2014), AOA oxidized NH_3 to NO_2^-, probably as a catalyst to NH_2OH oxidation (Vajrala et al., 2013). Ammonia-oxidizing archaea have also been shown to

produce N_2O from NO_2^- and of being capable of producing comparable N_2O yield as AOB from soil enrichment culture, in an incubation study (Wrage-Mönnig et al., 2018; Jung et al., 2014). However, higher N_2O emission produced from NO_3^- by AOB in soils have been observed, though AOA may be the dominant N_2O producers in unfertilized soils with low $NH4^+$ (Hink et al., 2018 ; 2017), and AOA contribution to N_2O production via nitrifier denitrification may be limited (Wrage-Mönnig et al., 2018; Gregorich et al., 2015).

2.2.4 Other microbial sources of N_2O emission in soils

Besides nitrification, denitrification and nitrifier-denitrification, other microbial sources like dissimilatory nitrate reduction to ammonium (DNRA or nitrate ammonification) (Baggs, 2011), anaerobic ammonium oxidation (Anammox) (Abed et al., 2013), anaerobic reduction of nitrate (Wrage et al., 2001; Wrage-Mönnig et al., 2018), fungal denitrification (Rohe et al., 2014; Maeda et al., 2015) and impact of soil inhabiting invertebrate activities (Kuiper et al., 2013; Nebert et al., 2011) also influence the production/consumption of N_2O emissions in soil.

2.3 Factors affecting N_2O emission in agricultural soils

Nitrous oxide emissions in agricultural soils are caused primarily by microbial nitrification and denitrification. Consequently, the factors that affect nitrification and denitrification processes can also determine N_2O emissions. These factors include edaphic factors (soil type, soil aeration, water content, pH, bulk density, carbon and nitrogen availability), environmental factors (temperature and rainfall) and human activities (fertilization, irrigation and residue addition) (Shang et al., 2016; Huang et al., 2015; Zhu et al., 2013). This research work will focus on soil water content and irrigation, nitrogen fertilizer addition and crop residue addition as drivers of N_2O emissions during canola production. Nitrogen fertilization and crop residue N provide readily available substrate for microbial activities. Soil water content affects water filled pore space (WFPS) and thus O_2 availability which is a strong determinant of N_2O production and consumption, particularly from denitrification. These factors play significant roles in the determination of microbial pathways of N_2O in soils, and understanding the pathways can help to formulate a mitigation strategy.

2.3.1 **Soil water content and temperature**

The predominant factors regulating N_2O production and the contribution of nitrification and denitrification to N_2O emission from soils, are soil water content and temperature (Liu et al., 2017). Soil water content, described as water filled pore space (WFPS), is inversely correlated with O_2 availability in the soil, and is frequently described as affecting soil N_2O emissions (Hu et al., 2015b; Kool et al., 2011). The diffusion of O_2 into the soil is affected by soil porosity and pore size distribution which regulates the WFPS, and plays an important role in soil aeration status (Butterbach-Bahl et al., 2013). The upsurge in WFPS due to wetting events such as irrigation, precipitation and snowmelt after prolonged dry soil conditions, facilitate soil nitrification. Avrahami and Bohannan (2009) observed positive responses of nitrification potential and nitrate concentration to increasing soil moisture content and fertilization. Denitrification is also favored by wetting events, and in doing so, promotes production of N_2O (Hu et al., 2015b). Lang et al. (2011) showed that N_2O production through nitrification was more sensitive to temperature change than NO_3^- production in agreement with other studies that showed that microbial N_2O production increased with temperature (Goodroad and Keeney, 1984; Sitaula and Bakken, 1993).

Huang et al. (2015) concluded that in N fertilized calcareous fluvo-aquatic soils, cumulative N_2O emissions were significantly correlated with WFPS, with 70% WFPS accounting for highest emissions. This was attributed to higher nitrifier denitrification (44-58%) and ammonia oxidation (35-53%) activities, with heterotrophic denitrification accountable for just (2-9%). When the soil is very dry nitrifier denitrification becomes a more active contributor (~29%) to N_2O emissions (Kool et al., 2010), while this pathway is not as active in wetter soils, contributing less than 3% of total soil N_2O emissions. At 45% WFPS ammonia oxidizers could contribute up to 88% of total soil N_2O emissions as observed by Well et al. (2008) in a ^{15}N-labeled microcosm study. Gödde and Conrad. (1999) reported that nitrification could contribute around 80% of total N_2O emissions depending on soil temperature and moisture

Soil moisture and temperature has been identified as the most sensitive factor to regulate O_2 availability in soil pores which determine the activities of nitrification and denitrification within the soil profile (Zheng et al., 2000). Soil water content and temperature not only determine the availability of O_2, but also affects the diffusion and transport of nutrients within the soil, and the metabolic activities of microbial cells(Hu et al., 2015c). This makes the relationship between

WFPS and rate of N_2O emissions complex, because of the heterogeneity of the soil in which anaerobic and aerobic conditions can exist simultaneously, relative to changes in WFPS as determined by soil water content (Hu et al. 2015a).

Soil moisture content is the principal factor regulating N_2O emission from soils (Liu et al., 2017). In a recent meta-analysis by Rochette et al. (2018), 38% of the variation in cumulative N_2O emission from soils was ascribed to growing season precipitation - the single factor that explained the greatest amount of variation in N_2O emission. However, at site level, management was observed to impact N_2O emission more than climatic factor (Congreves et al., 2016). In soil receiving N fertilizer, N_2O emissions were highly correlated to WFPS (Huang et al. 2015). Changes in soil water content plays a contrasting role on gaseous and liquid diffusion rates in the soil which consequently affects microbial activities by dictating O_2 availability, and microbial utilization of NO_3^- in the soil (Liu et al., 2017; Banerjee et al., 2016; Hu et al., 2015b). Soil moisture content affects microbial activities, but under very high moisture regime microbial activity can be inhibited (Signor et al. 2013) reducing N_2O production, or denitrification goes to completion which also reduces N_2O emission. Under moisture alternations between wetting, drying and static moisture conditions Banerjee et al. (2016) found microbial communities to be dependent on previous moisture regime, and concluded that previous soil moisture content before wetting or drying conditions affects microbial activities, transcription, composition and importantly, N_2O emissions.

Effects of irrigation practices.

In order to meet the increasing demand for food by the growing world population, more land needs to be cultivated, and this will increase pressure on resources needed for agricultural production (Trost et al., 2013). There is substantial opportunity to increase food production in the Canadian Prairies through expansion of irrigated crop production, with large irrigable land base and significant freshwater resources (David et al., 2018). Irrigated crop management involves the use of water resources and nutrients at rates exceeding requirements in dryland cropping systems, thus altering soil moisture and fertility regimes (David et al., 2018) and promoting microbial activities (Butterbach-Bahl et al., 2013), that can result in greater emission of soil-derived GHGs. Effect of irrigation on N_2O emission is assumed to be the direct effect on soil WFPS, which is expected to be greater in an irrigated system (Gregorich et al., 2015). Irrigation could be a key driver of soil water content and WFPS can be a good descriptor of soil redox potential and conditions that affect

soil mineral N transformations and consequently soil N_2O production (Rochette et al., 2018, 2008a; Linn and Doran, 1984). Under dryland conditions in the Canadian Prairies, generally low N_2O emissions with periodic high emission events at snowmelt (Yates et al., 2006; Lemke et al., 1998) have been observed. Differential emission of N_2O from irrigated and non-irrigated systems is a result of increased amount of available soil N in an irrigated system, rather than increased WFPS (Trost et al., 2013) or soil moisture status (David et al., 2018). Large emission following precipitation or irrigation events after spring fertilization have been observed and attributed to denitrification (Halvorson et al., 2010; Dusenbury et al., 2008), and more than half of total recorded N_2O losses may occur at higher soil water content during spring thaw events (Lemke et al., 1998).

2.3.2 Nitrogen fertilizers

The use of N fertilizers has led to an increase in grain yield production all over the world (Mueller et al., 2012; Galloway et al., 2008), and has also caused environmental pollution (Chen et al., 2014a; 2014b). Nitrogen fertilizer-derived losses via nitrification and denitrification are important consequences of low N-use efficiency and are a main source of increased atmospheric N_2O deposition (Cui et al., 2013; Aulakh et al., 2000). Improving agricultural N use efficiency will reduce gaseous N loss and consequently lead to reduction of environmental risk (Chen et al., 2013). To increase N availability for plant use over a long period of time and increase fertilizer N-use efficiency in agricultural systems, it is important to control the conversion of N fertilizer applied as ammonium (NH_4^+) and urea to the more mobile oxidized form of nitrate (NO_3^-) (O'Sullivan et al., 2011). Plant N use is controlled by several factors including: climate (temperature, precipitation) soil characteristics (texture, organic matter content, compaction, pH CEC), management practices (tillage, crop rotation, pest managements), and fertilizer application (Malhi et al., 2006, 2001).

The principle of 4R nutrient stewardship was adopted from best management practices (BMPs) to increase production and maximize profit for producers while addressing environmental concern (Bruulsema et al., 2015) and also present an opportunity to reduce N_2O emissions from agro-ecosystems (Burton, 2018). The rate, type, method and timing of N fertilizer application play a significant role in regulating N_2O emissions in agricultural soils (Signor et al., 2013). Rate of fertilizer application is influenced by many factors, including crop type and cultivar, inherent soil

fertility, and water regimes. One of the widely adopted ways of reducing N_2O emissions is reduction of N application rate, if rate of application exceeds crop requirements (Ma et al., 2010a; Grant et al., 2006; McSwiney and Robertson, 2005; Roy et al., 2014; Chantigny et al., 1998). The proportion of N_2O emissions and correlation with N fertilizer application is not well understood. In agricultural soils N_2O emissions are a result of microbial transformation of inorganic N. If the amount of N supplied is higher than crop N needs (Ma et al. 2010a; Millar et al. 2004), it will increase the potential for N_2O emissions with increasing availability of N (Daims et al., 2015). Nitrous oxide emissions increased exponentially with N rate (Cardenas et al., 2010) once the crop N needs had been met (Gregorich et al., 2015; Malhi et al., 2006; Groenigen et al., 2010). Other studies have observed a linear increase in N_2O emissions with N applications (Gao et al., 2013; Liu et al., 2012; Mosier et al., 2006; Helgason et al., 2005; Gregorich et al., 2005). Increase in N_2O emissions observed with higher N fertilizer application rate has been attributed to unused N (McSwiney and Robertson 2005; Grant et al. 2006).

Timing of N application to match crop requirement, is one of the greatest contributors to curbing N loss in agricultural system. Multiple applications of N to coincide with periods when plant demand is high can substantially increase N use efficiency (Robertson and Vitousek, 2009). Applying N fertilizer when the crop uptake is high can mitigate denitrification and, consequently N_2O emissions, by reducing nitrate concentration in the soil (Zebarth et al., 2012, 2008). Timing N application might pose a serious challenge, which can be overcome by split application of N or controlled release of N by adopting enhanced efficiency fertilizers or combination of these two approaches (Gregorich et al., 2015). Inconsistent results have been observed in the few studies available on split application of N in reducing N_2O emissions. In a two year study by Burton et al., (2008), split application of N resulted in reduction of cumulative N_2O emission by minimizing the supply of NO_3^- , when demand for terminal electron acceptor was high as a result of increased rainfall resulting in reduced aeration (Burton et al., 2008). However, split N application did not reduce N_2O emissions when Zebarth et al., (2012) compared controlled release of N using polymer coated urea (PCU), conventional fertilizer N management and split fertilizer N application on growing season N_2O emissions from rain-fed potato study. Nitrous oxide emissions depend on the availability of N in the soil and soil condition after N application. Rainfall events or increased moisture availability immediately after split application of N fertilizer at seeding can increase N_2O emissions (Tan et al., 2009). The type of fertilizer also influences the magnitude of N_2O emissions

(Signor et al. 2013). Ammonia fertilizers induce N_2O emissions at a slower rate than NO_3^- fertilizers (Signor and Cerri 2013), because ammonia fertilizers have to be nitrified before denitrification occurs and NO_3^- fertilizers can be denitrified immediately. Ammoniacal fertilizers, particularly urea, are used extensively by North America farmers because of their wide availability and low cost per unit of N, They can be easily manufactured, stored, transported, distributed and handled (Gagnon et al., 2012). However a considerable amount of N can be lost from urea through volatilization if it is not incorporated into the soil soon after application (Chen et al., 2008).

Urea and organic N hydrolysis in the soil.

Soil hydrolytic enzymes urease and arylamidase mediate hydrolysis of urea and N-terminal amino acids (peptide, amides or arylamides) respectively. The transformation and mineralization organic N plays a pivotal role in soil N-cycling (Shi et al., 2018; Fatemi et al., 2016; Jian et al., 2016). Enzyme synthesis and secretion are the main biotic processes influencing enzyme activities and are normally affected by substrate availability and nutrient concentration (Burns et al., 2013) in addition to abiotic factors, such as soil temperature, soil water content and pH (Wang et al., 2014; Alster et al., 2013). Elevated N deposition as a result of human activities has increased N availability, altered soil microbial biomass and consequently affected enzyme activities (Kamble and Bååth, 2016). These changes will affect ecosystem functions such as organic matter decomposition, nutrient cycling and plant-microbe interactions (Shi et al., 2018). The effect of increased N deposition on plant aboveground productivity and biodiversity loss (Farrer and Suding, 2016) and soil enzyme activities (Wang et al., 2014; Song et al., 2013; Lagomarsino et al., 2009) has been extensively studied, with inconsistent results attributed to differences in vegetation, soil types, form and level of N fertilization (Jian et al., 2016; Stursova et al., 2006).

2.3.3 Crop residue addition

Crop residue is defined as the non-edible plant parts that are left in the field after harvest (Lal, 2005). The global production of crop residue is estimated at almost 4 billion Mg, of which 74% are cereals, 8% legumes, 3% oil crops and 5% tubers (Lal, 2005). Application of crop residue to agricultural soil is important, as it provides readily available C and N, in addition to other nutrients (Kumar and Goh, 1999), resulting in soil fertility improvement and increasing organic C (Wang et

al., 2016). Globally about 12 Tg of N is added to the soil through crop residues (Cassman et al., 2002), and crop residue N concentration affects decomposition rate (Janzen and Kucey, 1988).

Crop residue addition to soil contributes to N_2O emissions, because of residue N content controls on mineralization/immobilization processes and, consequently N availability (Li et al., 2015; Gregorich et al., 2015; Doltra and Olesen, 2013), it influences denitrification rates and denitrifier abundance (Chen et al., 2013; McKenney et al., 1993; Aulakh et al., 1991). The magnitude and pattern of N_2O emissions varied among different crop residues (Li et al., 2015). Among the factors affecting N_2O emission from residue amended soil are quality (biochemical composition) and quantity of incorporated residue (Li et al., 2016b; Gregorich et al., 2015; Garcia-Ruiz and Baggs, 2007; Baggs et al., 2003). Residue N content and C:N ratios have been coupled with the magnitude of N_2O emissions. Residue with low C:N ratio is expected to increase N_2O emissions in residue amended soils (Li et al., 2016b). Application of crop residue with high C:N ratio stimulates microbial immobilization of N released during residue decomposition in soil, leading to lower N_2O emissions while application of N rich crop residue (low C:N ratio) can increase N_2O emissions in soils (Baggs et al., 2003; Millar et al., 2004). Applying residues with a high % N, can increase N_2O emission through the production of readily available NO_3^--N for denitrification during conditions of anaerobicity. Nitrogen concentration of major crops in western Canada are shown in Table 2.1. Lignin and polyphenols are recalcitrant compounds in plant residue that can affect the rate of residue decomposition and consequently affect soil N_2O emissions, thus soil N_2O emissions are not dependent only on C:N ratio of crop residue (Garcia-Ruiz and Baggs, 2007; Millar et al., 2004). Newly produced biomass from bacterial and fungal utilization of crop residues with varying C:N ratios may affect the dynamics of N_2O production (Rousk and Bååth, 2007; Vinten et al., 2002). Crop residues like wheat, with higher C:N ratio (97:1) compared to maize with lower C:N ratio (52:1) when amended with fertilizer led to a significant increase in N_2O emissions compared with maize residue amended with fertilizer (Gao et al., 2016). The higher emission from wheat was associated with lignin content which degrades at slower rate compared to maize residue, this might cause reduction in N immobilization from wheat residue (Begum et al., 2014), which may increase availability of N in the soil for denitrifiers and consequently increase in N_2O emissions (Gao et al., 2016).

Table 2.1 Nitrogen concentration in aboveground residue (AGR), belowground residue (BGR) and total residue (AGR + BGR) of major crops in western Canada (Janzen et al., 2003; Thiagarajan et al., 2018)

	N concentration (g N kg^{-1} dry matter basis)					
	AGR		BGR		AGR + BGR	
Crop	Janzen et al. (2003)	Thiagarajan et al. (2018)	Janzen et al. (2003)	Thiagarajan et al. (2018)	Janzen et al. (2003)	Thiagarajan et al. (2018)
Wheat	6	6.64	10	10.51	16	17.15
Oat	6	6.83	10	13.83	16	20.66
Barley	7	8.81	10	12.39	17	21.2
Dry pea	18	21.02	10	21.99	28	43.01
Lentil	10	11.72	10	nd†	20	nd
Canola	8	12.53	10	8.83	18	21.36
Flax	7	12.2	10	nd	17	nd
Alfalfa	15	13.8	15	18.17	30	31.97

† Not determined in Thiagarajan study.

Also affecting crop residue decomposition are: particle size of the residue, methods of application and environmental conditions (Frimpong et al., 2012; García-Ruiz et al., 2012; Garcia-Ruiz and Baggs, 2007; Aulakh et al., 2001; Kumar and Goh, 1999). Residue quality and type can affect following season N_2O emission. In their study, Lemke et al. (2018) observed higher N_2O emission from wheat grown on canola residue than wheat grown on pea residue in a rotation on the Canadian Prairies. Liu et al. (2011) also observed that application of wheat straw increased the cumulative N_2O and NO emissions in the following maize season, in a straw-amended wheat-maize rotation in China. Crop residue C:N ratio may not be a good predictor of N_2O emissions. Other chemical compounds or dissolved organic C (DOC) in crop residue and its different constituents may dictate N_2O emissions when crop residue is added to soils. For example, denitrification rate is significantly impacted by C availability (Tatti et al., 2017; Baruah et al., 2016; Huang et al., 2004; Azam et al., 2002). The influence of C on denitrification is, in addition to being a substrate for denitrifiers, C stimulates microbial metabolism, thus increasing oxygen (O_2) consumption and leading to the development of anaerobic microsites favorable for heterotrophic denitrification (Azam et al., 2002).

Crop residue type is expected to have a significant influence on N_2O emissions in the soil. Nitrous oxide emissions in the soil are regulated by inorganic N content, microbial activity, DOC concentration and temperature; all of which are affected by addition of crop residues (Arcand et

al., 2016; Turmel et al., 2015; Blanco-Canqui and Lal, 2009; Kumar and Goh, 1999). Several studies reported an increase in N_2O emissions after the addition of plant residues (Gao et al., 2016; Huang et al., 2004; Velthof et al., 2002) suggesting that decomposition of crop residues can provide C and N to microorganisms to stimulate microbial activity resulting in rapid increase in N_2O production (Azam et al., 2002). Chen et al., (2013) and Shan and Yan, (2013), showed positive correlations between crop residue-C, soil microbial respiration and N_2O emissions. Lemke et al., (2018) reported that total crop residue N did not explain higher N_2O emissions from the wheat phase of a canola-wheat sequence of a rotation in their study. Canola residue with lower total N compared with pea residue induced higher N_2O emission in a subsequent crop. Also the amount of residue left behind by canola and wheat were not significantly different from one another, and the amount of N supplied by the canola residue could not account for enhanced N_2O emission in the subsequent crop.

The influence of tillage on N_2O emission can be viewed as a cascade effect (Gregorich et al., 2015). Tillage affects soil pore distribution and consequently soil structure, aeration, soil temperature and water content (Gregorich et al., 2015). Residue decomposition rate, N mineralization and immobilization are affected by microbial activity, which is governed by soil aeration status, soil temperature, water content, and nutrient availability, among other factors. Tillage can cause increased evaporation and thus dry soil conditions. No-tillage can conserve soil water and lower soil temperature as a result of decreased soil disturbance and increased residue accumulation on the soil surface (Sainju et al., 2012; Al-Kaisi and Yin, 2005; Curtin et al., 2000). There are contrasting views about the effect of tillage practices on N_2O emissions. Some studies have shown increased emission with no till or reduced tillage (Ball et al., 1999), lower N_2O emissions (Lemke et al., 2011; Drury et al., 2006; Mosier et al., 2006) as a result of reduced soil disturbance and microbial activity, or no difference in emission due to tillage practice (Rochette et al., 2008b).

2.4 Soil microbial abundance, diversity and N_2O emissions

Soil microbes are important for soil and ecosystem functions. The relationship between biogeochemical processes and microbial diversity that control a process is of vital importance (Jones et al., 2013). Microbially-driven N conversions and resulting N_2O depend on the aeration status of the soil, availability of inorganic N substrate, labile organic C (Begum et al., 2014), temperature

and water availability (Gao et al., 2013). Nitrous oxide can be produced as a by-product of nitrification, or as an end-product or intermediate product of nitrite reduction during the denitrification process (Ishii et al., 2014). Hence, soil nitrifier and denitrifier abundance has a significant impact on N_2O emissions (Hu et al., 2015a; Baggs, 2011). The abundance and diversity of N_2O producers and consumers can increase or decrease as a result of long-term N fertilization (Gregorich et al., 2015). These changes are brought about due to the amount of residue derived C inputs, short-term concentration of N, change in soil pH as a result of increased use of N fertilizer and this strongly affects microbial abundance. Microbial community composition is strongly influenced by soil pH, thus the acidifying effect of N fertilization will influence N_2O emission through changes in the relative abundance of N_2O producers and consumers. Increase in AOB abundance with increasing soil pH has been documented (Hink et al., 2018), and these organisms may be absent in low pH environments (Prosser and Nicol, 2012). Type of N fertilizer can also play a role in microbial abundance and community composition (Gregorich et al., 2005). High concentration of N is toxic to microorganisms, ranging from a short lived effect of urea and ammonium salts (Geisseler and Scow, 2014) to long-term toxic effect of anhydrous ammonia fertilizer, and may result in decreased microbial biomass (Campbell et al., 2011). Substrate availability as a result of residue addition or removal can also dictate microbial response, abundance and diversity and consequently N_2O emissions. Organic or mineral N fertilizers, and crop residue decomposition, are likely to change denitrifier communities through effects on soil NO_3^- and O_2 availability and DOC (Duan et al., 2018; Tatti et al., 2015; Enwall et al., 2010). The effect of residue addition on microbial communities and response will be affected by other prevailing environmental and soil conditions. Pelster et al. (2013) showed that under freeze-thaw conditions residue return may decrease N_2O emission due to immobilization of N. However, Németh et al. (2014) observed increased N_2O emission as a result of crop residue removal, which was correlated with lower copy number and transcription of *nos*Z gene, suggesting incomplete reduction of N_2O to N_2, rather than increase in the amount of N denitrified (Gregorich et al., 2005). Increased microbial biomass was observed under long-term no-till (Helgason et al., 2009). Similarity in emission between tilled and no-till system observed by Six et al. (2004), may indicate improved soil structure/aeration, decreased anaerobic microsite and denitrifier abundance and consequently lower N_2O formation.

3. MICROBIAL PATHWAYS OF NITROUS OXIDE EMISSIONS IN IRRIGATED CANOLA (*BRASSICA NAPUS L.*)

3.1 Preface

Canola (*Brassica napus* L.) is an important crop in western Canada, and its cultivation depends heavily on nitrogen (N) fertilizer application. There is potential for increased canola production through irrigation in the Canadian prairie, with a large irrigable land base and plentiful water resources. However, it is important that we understand the implication on the environment. Irrigated cropping systems are usually characterized by increased application of N fertilizer, coupled with increased soil moisture content. These are factors driving nitrous oxide (N_2O) production in soils. This study investigated the impact of irrigation, increasing rate of N application and timing of N fertilizer application, either 100% broadcast at seeding or applied as a split rate (50% at seeding and 50% at bolting), in a two-year field study conducted at Canada-Saskatchewan Irrigation Diversification Centre (CSIDC) in Outlook, SK Canada.

3.2 Abstract

Nitrous oxide production in soils is due to the response of nitrifier and denitrifier communities to soil management practices. Baseline information on the response of these microbes in irrigated soils in western Canada, and particularly in irrigated canola is currently lacking or insufficient. This study investigated the impact of two different rates (110 and 220 kg urea-N ha^{-1}) and timing of N fertilizer application, either broadcast at seeding or applied as a split rate (50% at seeding and 50% at bolting) on N_2O emissions, also accounting for the effect of irrigation. Nitrification and denitrification potential, microbial enzyme activities responsible for key conversions of organic N (urease and arylamidase), and the abundance of genes involved in nitrification and denitrification (AOB *amoA*, AOA *amoA nirS*, *nirK*, and *nosZ*) were measured in soils in two consecutive years (2015 and 2016). We observed an increase in N_2O emission with increasing rate of N application, either applied 100% at seeding or as a split application (50% at seeding and 50% at bolting). Varying enzymatic response to N application was governed by differences in soil moisture content. Ammonia oxidizing bacteria (AOB) abundance increased with increasing rate of N application,

while ammonia oxidizing archaea (AOB) remained stable. Response within denitrifying community (*nirK*, *nirS* and *nosZ*) was only observable in 2016 with more evenly distributed precipitation. Our findings show that existing soil condition prior to management events affect magnitude and possibly pathways of N_2O emission in soils

3.3 Introduction

Nitrous oxide is a strong greenhouse gas (GHG) with approximately 300 times greater warming potential compared to CO_2 and is a main driver of ozone depletion in the stratosphere (Ravishankara et al., 2009). In Canada, 70% of annual N_2O emissions result from agricultural activities (Helgason et al., 2005), of which agricultural soils account for about 55% (Ellert and Janzen, 2008; Helgason et al., 2005). Nitrous oxide emission from soil is driven by the microbially-mediated processes of nitrification and denitrification, thus management practices that affect microbial abundance and activity can affect N_2O emissions (Butterbach-Bahl et al., 2013). Saskatchewan is the largest producer of canola in Canada, responsible for ~50% of canola production in 2018 (Statistic Canada, 2019). A lot of Saskatchewan agricultural land is in semi-arid climatic zones where precipitation is often limiting, thus creating potential for increased irrigated canola production. Irrigation as a management practice to increase production is often accompanied by N application at rates greater than applicable to rain-fed agriculture. This creates a greater potential for GHG emission from the soil because of altered soil temperature, moisture content, fertility regimes, soil microbial activities (David et al., 2018) and by altering soil capacity to act as sinks or sources of GHGs, especially, CO_2 and N_2O (Trost et al., 2013; Lal, 2004).

Higher N_2O fluxes are frequently observed after precipitation or irrigation events when there is available N in the soil, particularly following N fertilization (Gao et al., 2013; Ellert and Janzen, 2008), and during spring thaw (Lemke et al., 1998) when saturated conditions are more likely to occur. A mismatch between the time of N application and crop demand for N, for example N application at seeding (Crews and Peoples, 2004), can increase soil NH_4^+ and NO_3^- concentrations and lead to increased soil N_2O emissions (Gao et al., 2017). Thus, because of higher levels of N

fertilizer and the application of irrigation water, irrigated canola has high potential for N_2O emissions. Nitrogen turnover in the soil is a function of microbial abundance, substrate availability and environmental conditions controlling substrate availability and microbial activity.

Nitrous oxide can be produced as a by-product of nitrification, or as an end product or intermediate product of nitrate reduction by denitrifiers during denitrification (Ishii et al., 2014). Thus, the abundance of soil nitrifiers and denitrifiers can have significant impact on soil N_2O emissions (Chen et al., 2019). Two groups of microorganisms are largely responsible for gaseous N losses in soil: nitrifiers and denitrifiers. Their activity is controlled by interplay between the availability of oxygen (O_2), carbon (C) and N (NH_4^+ or NO_3^-) availability. Since soil moisture status largely determines O_2 and C availability, and since N additions are greater in irrigated systems, it is expected that these key groups of microorganisms function differently in irrigated vs. non-irrigated soils. By manipulating the timing of N application and irrigation events, N losses from nitrification and denitrification may be minimized, to reduce environmental impacts and improve the cost efficiency of N and water use. Some work has been done to study the loss of gaseous N in irrigated systems, however little is understood about physical, chemical or biological processes that drive these losses making it difficult to explain the variability that is often observed in N_2O emissions. For example work in eastern Canada by Rochette et al. (2010) determined that water and C availability are the dominant controls on N_2O emissions in irrigated vs. non-irrigated soils. In contrast, Ellert and Janzen (2008) observed that in western Canada, timing of irrigation often corresponded with peak periods of crop transpiration and de-coupled the application of water from N_2O emissions. Furthermore, a recent review by Trost, et al. (2013) concluded that in most cases higher N availability, rather than increased soil moisture, was responsible for increased N_2O emissions in irrigated soils. Thus, previous studies indicate that location and climate may have a dominant influence on the interaction of N_2O-generating process controls. Currently, there is no information available about the functioning of soil microorganisms in irrigated soils of western Canada. In order to interpret and understand the symptoms of N inefficiency (N_2O emissions) it is necessary to understand how soil microorganisms in Saskatchewan semi-arid soils respond to irrigated system management. By studying the microbial turnover of N in irrigated soils, my objective was to provide quantitative information about how chemical and biological factors interact to control soil N conversion enzyme activity, potential nitrification and denitrification activity, and N_2O emissions in Saskatchewan soils.

The specific objectives of this study were to (*i*) determine if different N fertilizer application rates and timing of application affects soil N cycling genes, N conversion enzymes activity and N_2O emissions in irrigated canola, (*ii*) relate N fertilizer application rates and timing of N fertilizer application with abundance of N cycling gene copies and N_2O emissions in irrigated canola. I hypothesized that (i) soil N conversion enzyme activity and N-cycling gene abundance will increase with an increase in the application rate of fertilizer N, and (ii) spring application of fertilizer N has a greater impact on soil N-conversion enzyme activity and N-cycling gene abundance than an in-season application of N.

3.4 Materials and Methods

3.4.1 Site description and experimental design

The experimental site is located at the Canada-Saskatchewan Irrigation Diversification Centre (CSIDC), Outlook, SK (51º 28‘ 31" N, 107º 3‘ 9" W) on a dominantly non saline calcareous Bradwell sandy loam soil. The soil pH was high at ~8, and with a soil organic matter range of 1.2-1.5% at 0-15 cm for the two fields in the study. Historical weather data for the Outlook station was downloaded from Environment Canada for 2015 and 2016. Mean daily temperature and precipitation were calculated from the data.

The experimental design is a randomized complete block design (RCBD), with five N treatments replicated four times (Appendix, Fig. A1). The experiment focused on two N levels, 110 and 220 kg N ha^{-1} applied as urea (46-0-0), either 100% broadcast and incorporated at seeding (110 and 220 kg N ha^{-1}) or split to apply 50% at seeding (55kg N ha^{-1} and 110 kg N ha^{-1}) and 50% pre-bolting (55kg N ha^{-1} and 110 kg N ha^{-1} - top dress application). The fifth treatment was an unfertilized control (0 kg N ha^{-1}). To describe these treatments (Rate, Timing and Time of sampling events) unique codes listed in Table 3.1 was utilized.

Table 3.1 List of treatment identifications and definitions

Treatments ID	Definitions
Rate of N application	
Control	Unfertilized treatment
BC-110	110 kg N ha^{-1}, all applied at seeding
BC-220	220 kg N ha^{-1}, all applied at seeding
Split-110	110 kg N ha^{-1}, 55 kg N ha^{-1} applied at seeding and 55 kg N ha^{-1} at bolting
Split-220	220 kg N ha^{-1}, 110 kg N ha^{-1} applied at seeding and 110 kg N ha^{-1} at bolting
Timing of N application	
BC	100 % N application at seeding
Split	50% N application at seeding, 50% at bolting
Time of sampling events	
Pre-SD	Pre-seeding
Post-SD	Post-seeding
Pre-IRR	Pre-irrigation
Post-IRR	Post-irrigation
Pre-BF	Pre-bolt fertilization
Post-BF	Post-bolt fertilization

3.4.2 **Soil sampling**

Soils were sampled before and after N application at seeding, and at the first major moisture event (irrigation) and when the crop reaches the bolting stage. Soil samples were collected as shown in Table 3.2.

Table 3.2 Soil sampling schedule

Events	**2015 Sampling dates**	**2016 Sampling dates**
Pre-seeding	May 11, 2015	May 17, 2016
Post-seeding	May 13, 2015	May 19, 2016
Pre-irrigation	May 19, 2015	June 7, 2016
Post-irrigation	May 21, 2015	June 9, 2016
Pre-bolt fertilization	June 23, 2015	June 21, 2016
Post-bolt fertilization	June 24, 2015	June 23, 2016

Soil samples was taken at a depth of 0-10 cm in 2015, and 0-7.5 cm in 2016 using a 3.175 cm dia. Backsaver probe (n=6 per plot). Samples were bulked to make a composite sample for each field plot. Soil samples were immediately transported from Outlook to Saskatoon on ice in a portable cooler. The soil was sieved using 4 mm mesh size sieve and a sub-sample stored at -80°C for DNA analysis, urease enzyme activity and arylamidase enzyme activity. For potential nitrification and denitrification enzyme activity assays, soil samples were stored at 4°C and the assay performed within four days. Soil gravimetric moisture content was determined by placing 10 g of fresh soil in a conventional oven for 24hrs at 105°C. Exchangeable NO_3^- and NH_4^+ were extracted from 5 g of soil by shaking in 50 mL of 2.0 *M* KCl (ratio 1:10). NO_3-N in the extract was determined using segmented flow analysis (cadmium reduction procedure) and determination of NH_4-N in the extract by segmented flow autoanalyzer (Technicon Instrument Corporation) indophenol blue procedure (Phenate method), as described in Soil Sampling and Methods of Analysis, Carter and Gregorich (2008).

3.4.3 N_2O emissions measurement and estimations

Greenhouse gas fluxes from all treatments were measured using square 25.4 x 25.4 x 15.0 cm vented, non-flow through, non-steady state chambers made from 6 mm thick clear poly methyl methacrylate (PMMA, "acrylic'') (Fig. 3.1). A GHG chamber base was installed 5 cm into the soil on each plot, yielding a headspace volume of 6.45 L over an area of 645 cm^2. The chambers were placed between seed rows and kept in place throughout the growing season. To give a similar plant density as the rest of the plot, canola was hand seeded along the outside of the two sides of the chamber parallel to the seed row. When the plots were sampled, the bases were covered with an insulated PMMA lid, which allowed the gases to accumulate; gas samples were then collected at a single time point 20 min. after the chamber lid was placed on the base. A 20 mL disposable syringe fitted with a 22-gauge needle was inserted through a rubber septum in the chamber lid for gas sampling. The sample was drawn into the syringe, and injected into pre-evacuated 12 mL Exetainer® vials (Labco Limited, UK), and transported to Saskatoon for analysis at the University of Saskatchewan's Prairie Environmental Agronomy Research Laboratory (PEARL). Four ambient air samples were also taken to represent time zero. Manual sampling conducted before, on the day of and after seeding, fertilizer application and irrigation events was used to assess the temporal

influences of N fertilizer treatments and irrigation on N_2O emissions. The emissions were combined to best capture the influence of sampling event conditions on N_2O emissions. This approach was taken to capture the lag occurring between a change in conditions and the realization of the microbial response.

Fig. 3.1 Photo showing (A) the MFC-GC manual gas sampling base and (B) the AFC-FTIR automated gas sampling chamber in the field.

3.4.4 **Soil microbial enzyme activity assays**

Potential enzyme activity assays were conducted for arylamidase (hydrolysis of N-terminal amino acid from arylamides) and urease (conversion of urea to NH_4^+) according to methods described by Dick (2011). Potential nitrification (conversion of NH_4^+ to NO_3^-) assay was performed at each sampling time while denitrification enzyme activity (conversion of NO_3^- to N_2) assays were performed at selected sampling times (before seeding, before irrigation, before and after the split fertilizer application). However, in 2015 data were not collected for arylamidase, denitrification enzyme activity (DEA) and urease enzyme activities after irrigation. Briefly, the arylamidase enzyme assay was conducted on three technical replicates and one control for each sample. One gram of soil was incubated with 0.1 M THAM buffer pH 8.0 at 37°C for 1 h, and colorimetric determination of β-naphthylamine produced by arylamidase activity was measured using a spectropho-

tometer at 540 nm (Acosta-Martinez and Tabatabai, 2000a). Urease enzyme activity was quantified by colorimetric determination of NH_4^+ released when 5 g (n=3) of the soil sample was incubated with 20 mL 0.1 M Borate buffered urea solution for 2 h at 37°C, the blue color developed by the addition of 2 mL sodium dicloroisocyanurate was measured using a spectrophotometer at 660 nm (Kandeler and Gerber, 1988)

Potential nitrification was determined by adding 100 mL working solution containing 1.5 mM NH_4^+ and 1.0 mM PO_4^{3-} to 15 g of field moist soil. Soil slurry formed was shaken for 24 h on a rotary shaker at 180 rmp at 20 °C, and 10 mL subsample removed at intervals of 2, 6, 20 and 24 h. The 10 mL soil slurry sampled was filtered using pre-rinsed Whatman No. 2 filter paper, and analyzed for NO_3^- on an autoanalyzer, using the cadmium reduction method (Tel and Heseltine, 1990).

Potential denitrification rate was measured using the acetylene inhibition method, involving short-term incubation under anaerobic conditions and the soil supplied with NO_3^- and available C source described by Luo et al. (1996). Two technical replicates per sample were prepared by weighing 10g dry soil (stored within five days (d) of field sampling). DEA solution containing 2.8 *m*M glucose,.1 *m*M KNO_3 and 125µg chloramphenicol was added to each microcosm and sealed. The microcosms were flushed with N for 2 min. Gas sampling was done at times 0, 30, 60 and 90 min. At time 0, 10 mL acetylene was injected into the microcosm and mixed for 15 s, by slowly moving the syringe plunger, this is to block N_2O conversion to N_2. At the last 5 s of mixing, 10 mL of gas was drawn into a pre-evacuated 12-mL Exetainer vial (Labco Inc.; Ceredigion, UK). With a new syringe,10 mL of N_2 gas was injected to replace the gas sampled from the microcosm, and the microcosm placed on a rotary shaker at 125 rpm until T30 (30 min), and 10 mL of gas sampled from the microcosm into the prepared evacuated vial. The gas sampled was replaced with N_2 gas and the microcosm returned to the rotary shaker until the T60 gas sampling, and the procedure repeated till T90 gas sampling.

3.4.5 **N-cycling gene abundance**

Extraction of DNA and qPCR. DNA was extracted from 0.25g of soil using DNeasy® PowerSoil® Pro kit (QIAGEN, Strasse, Germany) according to the manufacturer's instruction. DNA

extracts were stored at -80°C (Smith et al., 2010). Prior to quantitative PCR of archaeal and bacterial *amoA, nirS*, *nirK, nosZ* clade I and *nosZ* clade II measurement, genomic DNA template concentration of the samples were adjusted to 10 ng μL^{-1} after measurement with Qubit®2.0 Fluorometer with Qubit® dsDNA HS assay kit (Thermo Fisher Scientific, Waltham, MA USA).

The qPCR products for archaeal and bacterial *amoA, nirS*, *nirK*, and *nosZ* clade I were amplified with an ABI StepOnePlus Real-time PCR system (Life Technologies, Burlington, ON) using SYBR green detection chemistry. Standards were prepared using an Invitrogen TOPO TA Cloning Kit (Life Technologies, Carlsbad, CA) to insert amplified target template into the vector. Colonies successfully cloned with the insert (white coloration) were cultured using liquid broths, following the protocol in the TOPO TA Cloning® kit, incubated at 37°C for 16 h. Qiagen QIAprep Spin Miniprep kit (Qiagen, Mississauga, ON) was used to extract DNA (plasmid with insert) from the culture, the plasmid linearization was done using Hind III restriction enzyme (Life Technologies, Burlington, ON) and run on a 1% agarose gel. The bands with linearized products were excised and the gel purified. Qubit® 2.0 Fluorometer with Qubit® dsDNA HS assay kit (Thermo Fisher Scientific, Waltham, MA USA) was used to quantified DNA for the gel purification. A 10-fold dilution standard ranging between 10^2 and 10^9 gene copies was prepared with appropriate primers to target key genes in the nitrification (archaeal and bacterial *amoA*) (Liesack et al., 1997; Stephen et al., 1999) and denitrification pathways (*nirS*, *nirK* and *nosZ* clade I) (Throbäck et al., 2004). Gene quantification was done using an ABI StepOnePlus Real-time PCR system (Life Technologies, Burlington, ON), and quantification of each sample performed in triplicate in 96 well plates. Detailed description of reaction mixtures**,** thermocycler conditions and primer used can be found in appendix A1 and Table A1. Quantitative PCR efficiency (E) and the correlation coefficient (r^2) were determined based on the slopes of standard curves generated using serial 10-fold dilutions of DNA standards. All qPCR efficiencies were calculated as follows: $E\ (\%) = (10^{-1/\text{slope}} - 1) \times 100$. A seven-point standard curve, environmental samples and negative controls (no template DNA) were run in triplicate. The overall reaction efficiency was 82% to 85% for AOA *amoA* and AOB *amoA* qPCR assay, 85%-90% for *nos*Z, 90% -95% for *nir*K and *nir*S.

3.4.6 Statistical analysis

All statistical analyses were performed with IBM SPSS statistics v. 26. A general linear model for repeated measures analysis was used to test for significant difference in rates of N application, timing of N applications and time (sampling event) as fixed factors, on potential nitrification, arylamidase, urease enzyme activity, denitrifying enzyme activity, ammonium nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N), N-cycling functional genes (AOA *amoA*, AOB *amoA*, *nirS, nirK and nosZ*) and N_2O emissions. Sphericity of variance was tested using Mauchly's test and means compared using LSD post-hoc for multiple comparisons. Pearson correlation analysis was used to evaluate correlations between N_2O emissions, denitrifying enzyme activities, potential nitrification and number of gene copies present for microbial denitrification and nitrification at $p < 0.05$.

3.5 Results

3.5.1 Weather conditions and nitrous oxide emissions

In 2015, daily mean temperature ranged from 5.1ºC to 23.9ºC and daily precipitation was between 0 and 11.0 mm (Fig. 3.2). While in 2016, there was less fluctuation in the daily mean temperature, the range was between 11.1 ºC to 23.4 ºC and daily precipitation was more evenly dispersed between 0 and 8.7 mm (Fig. 3.3).

The daily N_2O flux sampled between May 10th (130 day) and June 29th (180 day) in 2015 showed a low emission of 2.4g N_2O-N ha^{-1} day^{-1} from the control treatment and high emission of 97.6g N_2O-N $ha^{-1}day^{-1}$ from BC-220 (Fig. 3.2). Daily N_2O flux between May 16th (day 137) and June 23rd (day 175) ranged from 2.1 g N_2O-N $ha^{-1}day^{-1}$ in the control (0 N) and 103.6 g N_2O- $ha^{-1}day^{-1}$ from BC-220 in 2016 (Fig 3.3).

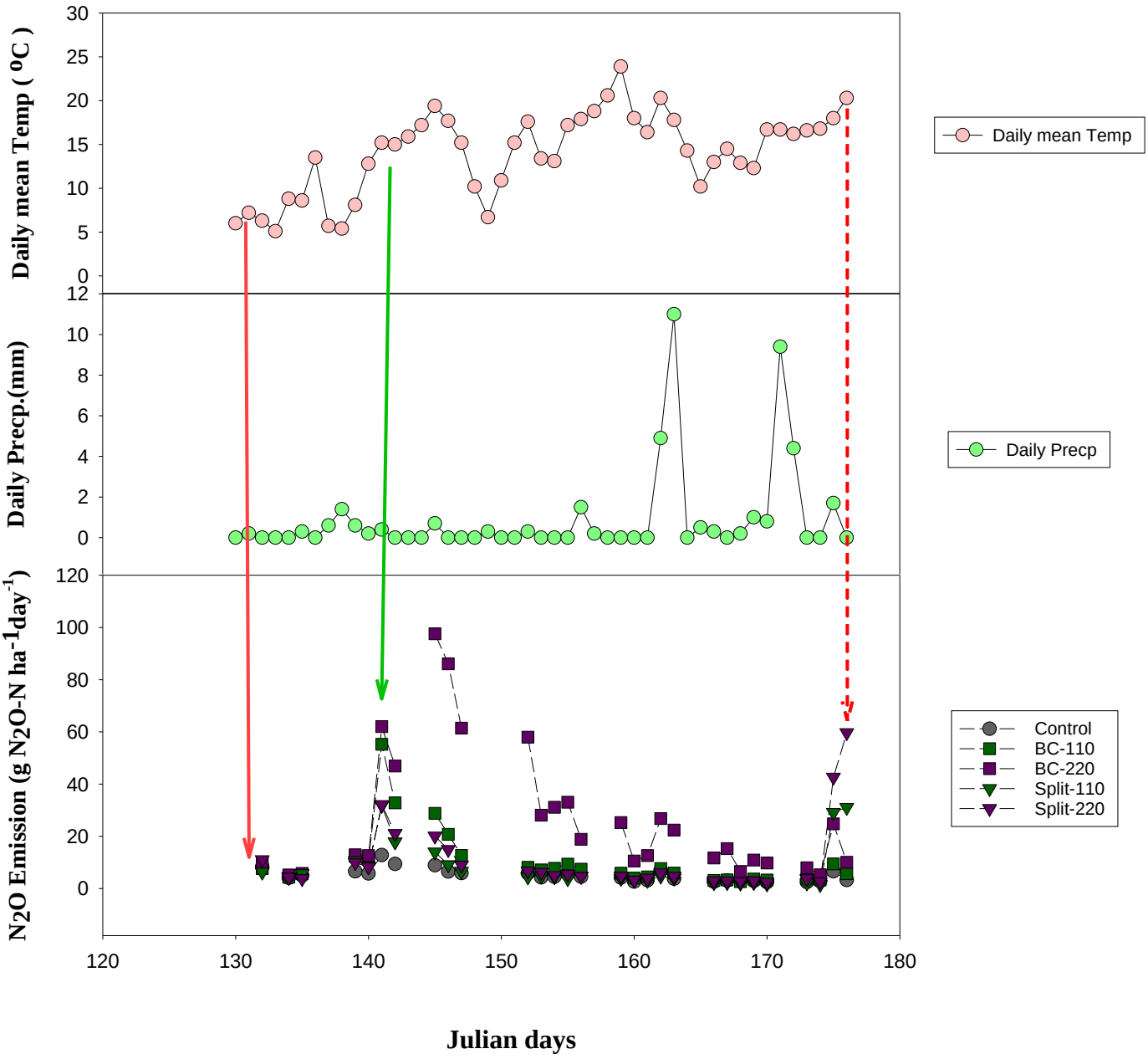

Fig. 3.2 Daily N_2O emission, mean temperature, daily precipitaion and sampling events in 2015 *(red arrow* first fertilizer application, *green arrow* irrigation application, and *dotted arrow* split fertilizer application)

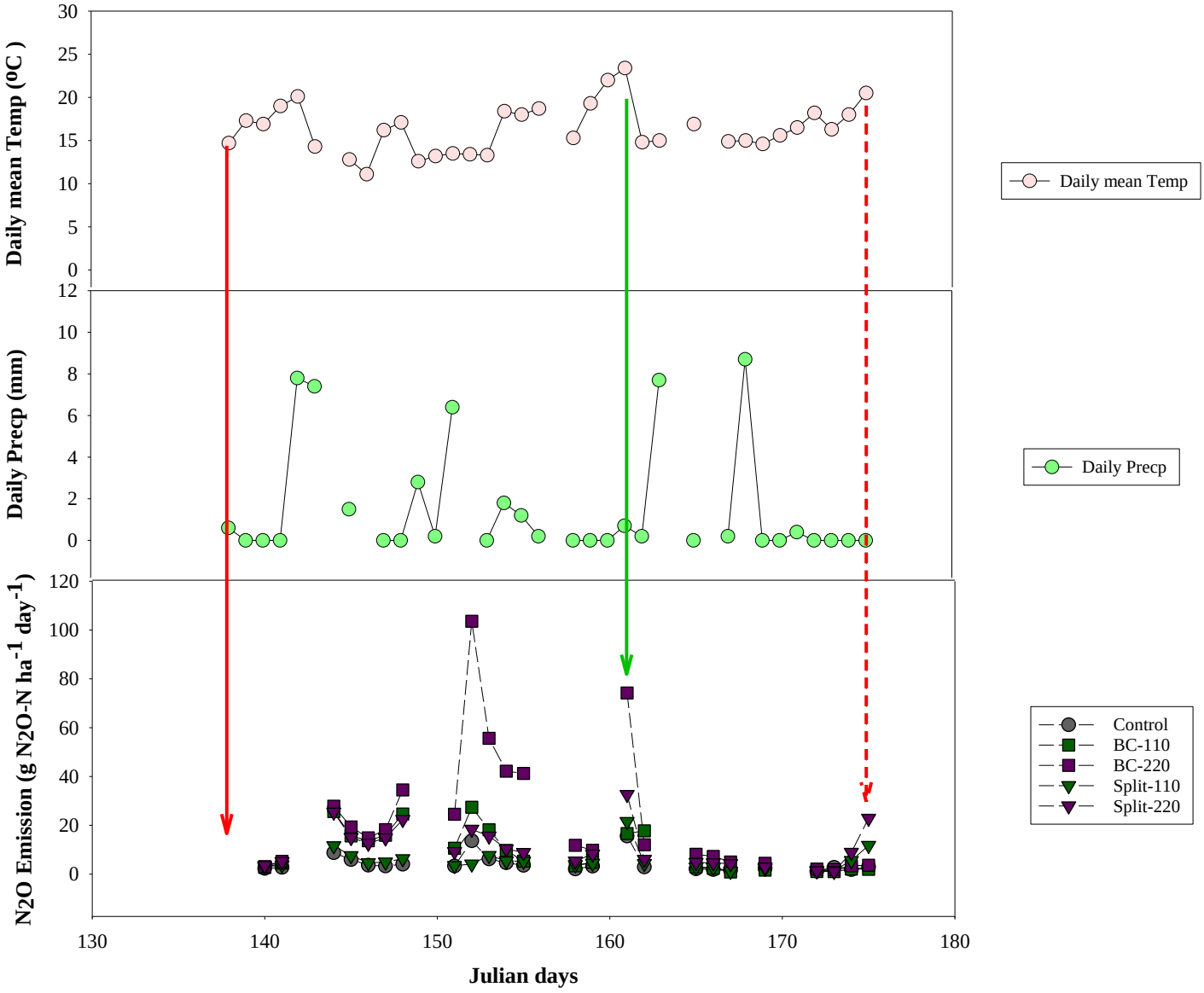

Fig. 3.3 Daily N_2O emission, mean temperature, daily precipitaion and sampling events in 2016 *(red arrow* first fertilizer application, *green arrow* irrigation application, and *dotted arrow* split fertilizer application)

3.5.2 **Nitrous oxide emission and soil moisture**

The lowest mean cumulative N_2O emissions were observed in the control treatment in 2015 and in control, BC-110 and Split 110 in 2016 (Table 3.3). However, it should be noted that the 2016 estimate did not include emission data before seeding (Fig 3.3 and 3.4). In 2015 N_2O cumulative flux within sampling periods on all treatments ranged between 4.1 g N_2O-N ha^{-1} from control treatments to 108.9 g N_2O-N ha^{-1} from the BC-220. In 2016 cumulative fluxes ranged between 2.1 g N_2O-N ha^{-1} from BC-110, to 86.2 g N_2O-N ha^{-1} from BC-220 (Fig. 3.4). In 2015 the lowest emission was recorded on soil without N applied sampled immediately after seeding, while the highest N_2O emission was observed on BC-220 sampled immediately after an irrigation event and Split-220 sampled after bolt fertilizer application. However, in 2016, the N_2O emission observed on control, BC-110, and Split-110 were not significantly different (p = 0.05) and highest N_2O emission observed on BC-220 sampled after an irrigation event (Fig 3.4). In 2015 mean cumulative emissions trend was control < Split-110 = BC 110 < BC-220 = Split-220, while in 2016 the mean cumulative emission trend was control = BC-110 = Split-110 < BC-220 = Split-220 (Table 3.3). Soil moisture content was not affected by rates of N application in either 2015 nor 2016 (Table 3.3). However, at sampling events, soil moisture was significantly (p = 0.05) different between management practices (Table 3.3).

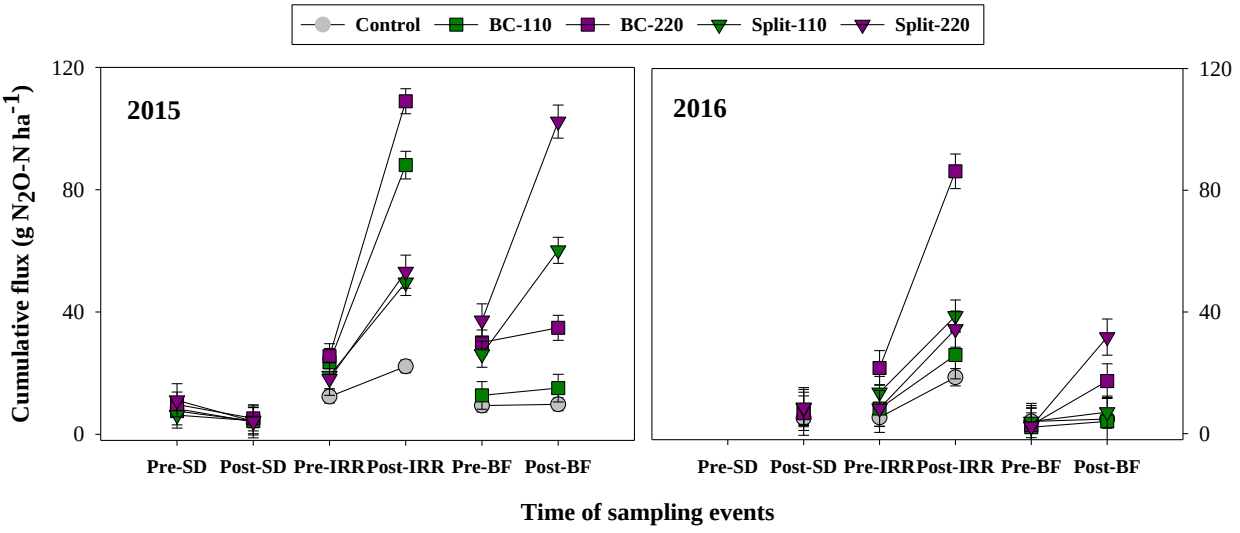

Fig. 3.4 Effect of nitrogen application rate, the timing of application and sampling event on N_2O emissions in irrigated canola.

Table 3.3 Repeated measures analysis of variance (ANOVA) and effects of rates and timing of N application and sampling events on cumulative N_2O emission and gravimetric water content (GWC) in irrigated canola.

Treatments	2015		2016	
	Mean cumulative flux (g N_2O-N ha^{-1})	GWC (gH_2O g^{-1} soil)	Mean cumulative flux (g N_2O-N ha^{-1})	GWC (gH_2O g^{-1} soil)
Rate				
Control	11.04c†	0.17	7.56b	0.14
BC-110	25.24ab	0.17	11.22b	0.15
BC-220	35.72a	0.16	25.44a	0.15
Split-110	28.41b	0.15	12.19b	0.14
Split-220	39.02a	0.16	19.00a	0.15
Timing				
Control	11.04b	0.17	7.56b	0.14
Broadcast	24.00a	0.16	14.74ab	0.15
Split	26.16a	0.16	12.91a	0.14
Time (Sampling event)				
Pre-SD	8.49cd	0.16b	---	0.15bc
Post-SD	4.30d	0.17ab	6.28b	0.13c
Pre-IRR	16.46c	0.13b	8.70b	0.10d
Post- IRR	45.66a	0.17ab	30.87a	0.17ab
Pre-BF	18.42bc	0.14b	3.49b	0.10d
Post-BF	29.06b	0.21a	9.35b	0.20a
Rate	***‡	NS¶	***	NS
Timing	***	NS	**	NS
Time	***	***	***	***
Rate X Timing X Time	***	NS	***	NS

† Different letters within the same column indicate a significant difference between means.

‡ *, **, *** Significant at $p \leq 0.05$, 0.01, and 0.001, respectively.

¶ Not significant.

3.5.3 **Soil ammonium and nitrate (NH_4-N and NO_3-N)**

In 2015 NH_4-N and NO_3-N were not quantified. In 2016 the highest level of soil NH_4-N was recorded after the first fertilizer application on treatments receiving single broadcast application of 220kg N ha^{-1} (BC-220) followed by the Split-220 treatment (Fig 3.5). As the NH_4-N in the soil declined, a corresponding increase was observed in NO_3-N, with the highest level of NO_3-N measured on BC-220 after irrigation. Increased NO_3-N was also observed on Split-220 in soil sampled after bolting application of N. There was a significant effect of rate and timing of N application, and also significant interaction with the time of sampling $p < 0.001$(Fig. 3.5).

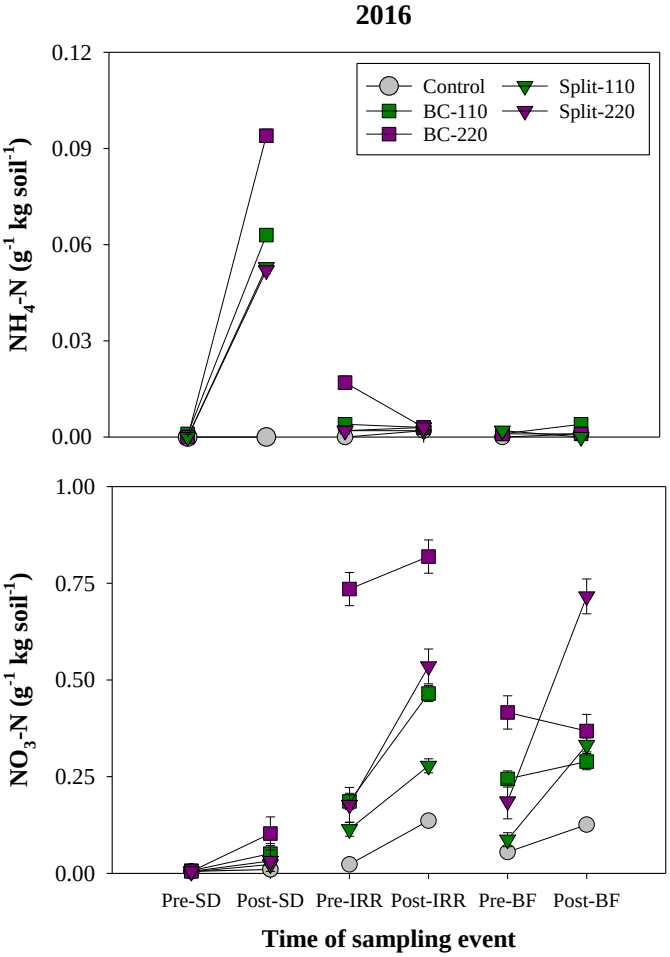

Fig. 3.5 Effect of nitrogen application rate, timing of application and sampling event on soil NH_4^+-N and NO_3^-N in irrigated canola**.**

3.5.4 **Microbial enzyme activities**

Enzyme assays conducted on soil samples collected from experimental plots in the year 2015 and 2016, shows that rate of fertilizer application and time of sampling events have a significant effect on N-conversion enzyme activities ($p < 0.005$) (Table 3.4). However, in 2015 the rate of N application had no significant effect on DEA ($p = 0.73$) and urease enzyme activity ($p = 0.32$). The timing of N application did not significantly affect DEA ($p = 0.75$) and urease enzyme activity ($P = 0.50$) (Table 3.4). Repeated measures ANOVA revealed that interaction between rate, timing and time of sampling event had a significant effect on urease enzyme activity ($p = 0.05$) in 2015 but not in 2016 ($p = 0.13$). There was not an interaction effect on DEA ($p = 0.88$) in 2015 and there was a 3-way interaction in 2016 ($p = 0.03$). Arylamidase and potential nitrification were significantly affected by the 3-way interactions ($p < 0.01$) in both 2015 and 2016.

Denitrifying enzyme activity (DEA). The effect of rate of N applications was not significant in the two years of the experiment on DEA ($p = 0.73$ and $p = 0.55$ in 2015 and 2016 respectively), while the timing of N application was only significant in 2016 ($p = 0.02$) (Table 3.4). In 2015 DEA was lowest in Split-110 and not significantly different from BC-220. In 2016 DEA was lowest in BC-220 (Fig. 3.6).

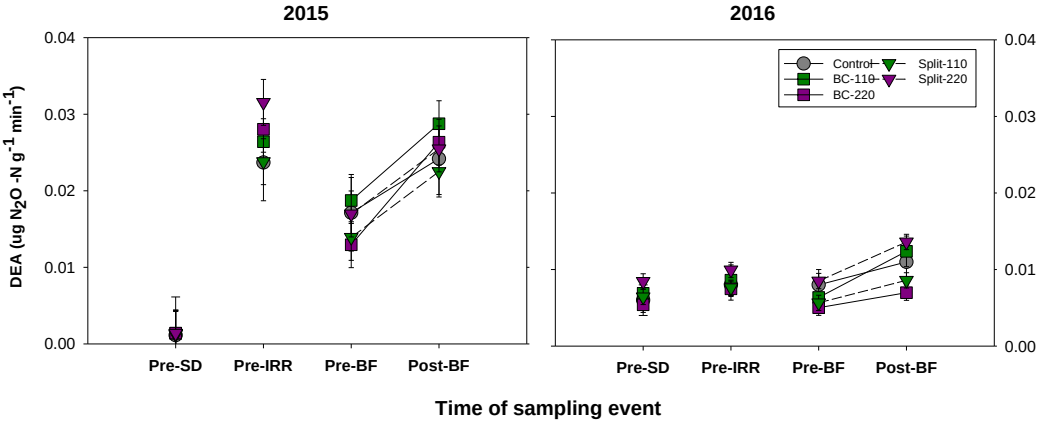

Fig. 3.6 Effect of N application rate, the timing of application and sampling event on soil denitrification enzyme activity in irrigated canola.

Table 3.4 Repeated measures analysis of variance (ANOVA) of enzyme activities at five rates and two-timings of N application at six sampling events in irrigated canola.

		2015[†]				2016			
		DEA[‡]	**Urease**	**Arylamidase**	**P. nitrification**	**DEA**	**Urease**	**Arylamidase**	**P. nitrification**
Rate	F	0.33	1.18	4.55	210.27	0.62	9.79	10.49	960.27
	p	0.73	0.32	*0.02*	*<0.01*	0.55	*<0.01*	*<0.01*	*<0.01*
Timing	F	0.30	0.71	22.50	203.61	4.40	8.43	7.38	1247.73
	p	0.75	0.50	*<0.01*	*<0.01*	*0.02*	*0.01*	<0.01	*<0.01*
Time (sampling event)	F	13.18	3.11	1009.30	18.71	2.11	3.73	19.46	249.91
	p	*<0.01*[¶]	*0.05*	*<0.01*	*<0.01*	0.15	*0.02*	*<0.01*	*<0.01*
Rate*Timing*Time	F	0.56	2.54	5.09	6.81	2.19	1.46	2.00	109.39
	p	0.86	*<0.01*	*<0.01*	*<0.01*	*0.03*	0.13	*0.02*	*<0.01*

[†] 2015 - Arylamidase and urease enzyme activity (n=100)

[‡] DEA = Denitrification enzyme activity, P =potential nitrification

[¶] Data in italics indicate that the measured enzymes activity were significantly ($p < 0.05$) affected by rates, timing and sampling events or [Rates x Timing x Sampling events]

Arylamidase enzyme. In 2015, arylamidase enzyme response to the rate of urea application (BC-110 and BC-220) was not significantly different from control (0 N). The treatments receiving split N applications (Split-110 and Split-220) showed a significantly higher enzyme activity compared to both control and plots receiving single fertilizer applications (Fig. 3.7). In 2016 BC-220 and Split-110 were not significantly different from each other and showed lower enzyme activity response compared to all other N application treatments. In 2015 arylamidase response to split application of urea was significantly higher than the response to the broadcast application and control, though the enzymatic response was lower on the broadcast application but not statistically different compared to the control. In 2016 there was no significant difference between broadcast and split application of urea, but all N applications were different from the control (Fig. 3.7). Time of sampling events was different in 2015 vs. 2016, with higher enzyme response to urea application immediately after seeding.

Urease enzyme. Urease enzyme activity responded to the rate of N-application only in 2016 ($p < 0.01$), with control and BC-110 having higher activity than other rates of N application (control = BC-110 > BC-220 = Split-110 = Split-220) (Fig. 3.7). Urea either applied 100% at seeding or 50% at seeding and 50% at bolting were not significantly different in 2015 ($p = 0.17$) nor 2016 ($p = 0.06$). However, in 2016 plot not receiving fertilizer had significantly higher urease activity ($p < 0.01$).

Potential nitrification. Nitrification potential was consistently higher in all treatments receiving urea compared with the control. In 2015 BC-220, Split-110 and Split-220 were not different from one another but were greater than BC-110 (Fig. 3.7). In 2016, all the rates of N-application were higher than the control (BC-220 > BC-110 > Split-220 > Split-110 > Control) (Fig. 3.7). Timing of N-application was significantly different between the treatments although the trend observed was opposite in the two years.

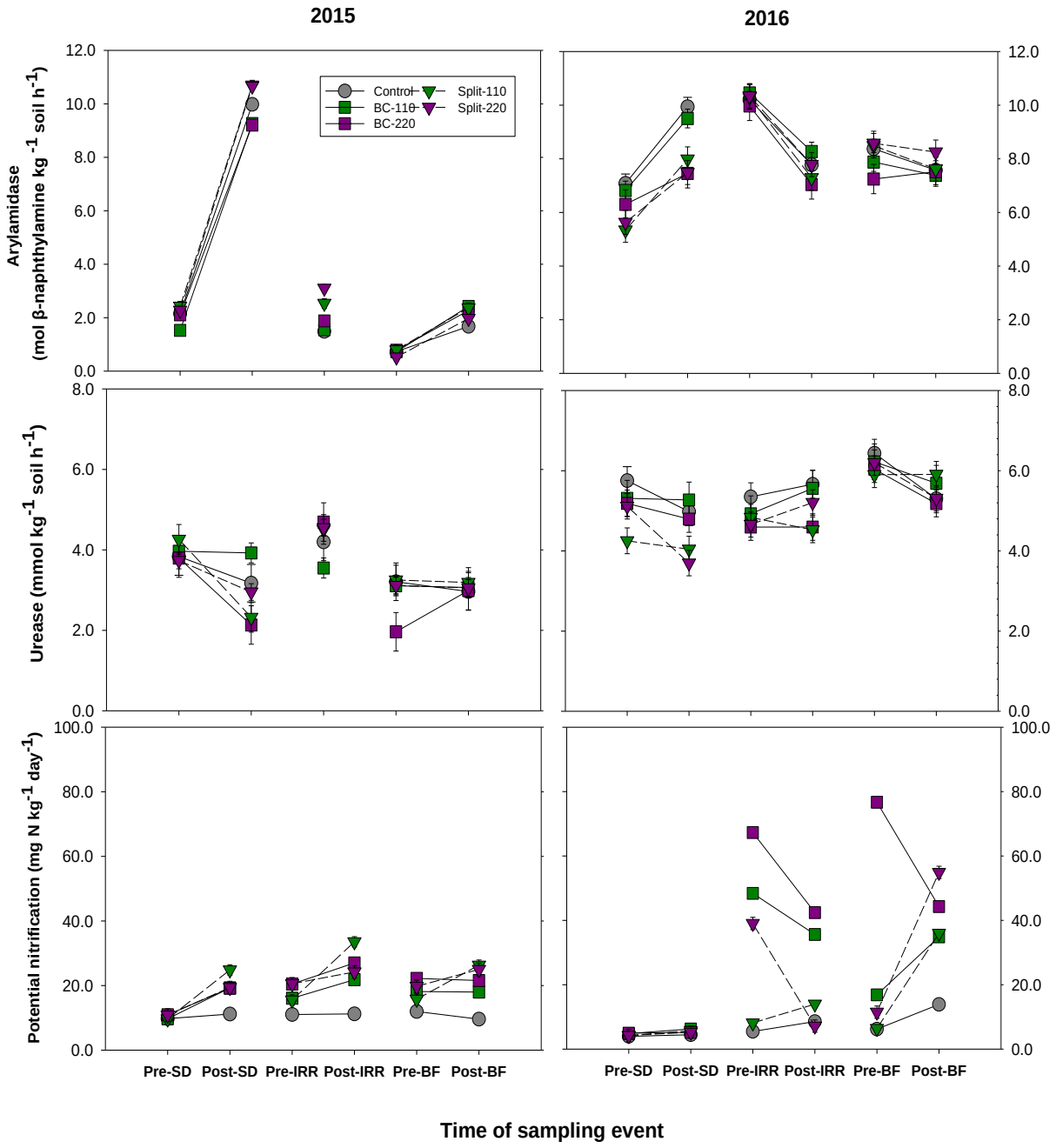

Fig. 3.7 Effect of N application rate, timing of application and sampling event on soil arylamidase enzyme activity, urease enzyme activity and potential nitrification in irrigated canola.

3.5.5 Nitrifier and denitrifier abundance

Real-time PCR was used to quantify the abundance of N cycling gene copies by a culture-independent analysis. The mean archaeal ammonia oxidizer (AOA) *amoA* gene copy abundance in all the rates and timing of N applications for the two-year sampling periods were higher than those of bacterial ammonia oxidizer (AOB) *amoA*, ranging between 1.5×10^9 and 2.0×10^9 copies g^{-1} dry soil (Table 3.5). The repeated measure ANOVA shows that the effects of both the rate and timing of N application were significant on AOB *amoA* gene copy abundance in the two years (Table 3.5) ($p < 0.05$), and the only N application rate difference was between BC-220 (220 kg N ha^{-1}) and Control (0 kg N ha^{-1}) (Table 3.5). There was no effect of rates and timing of N applications on *nir*S and *nir*K gene copy abundances in 2015, but BC-110, BC-220 and Split-220 significantly ($p < 0.05$) increased *nir*S gene copy in 2016 (Table 3.5). Nitrous oxide reductase is encoded by the *nos*Z gene that converts N_2O gas to N_2. The ratio of *nos*Z to AOB *amoA* was significantly higher in the control vs. fertilized treatments ($p < 0.05$). Repeated measure ANOVA of sampling event showed a significant difference ($p < 0.05$) between the management practice of fertilizer application and water irrigation in both 2015 and 2016 (Table 3.5).

Table 3.5 Repeated measures analysis of variance (ANOVA) and effects of rates and timing of N application and sampling events on N-cycling genes in irrigated canola.

Treatments	2015					2016				
	AOB *amoA*	**AOA *amoA***	***nir*S**	***nir*K**	***nos*Z**	**AOB *amoA***	**AOA *amoA***	***nir*S**	***nir*K**	***nos*Z**
	…… log gene copies g^{-1} soil ……					**…… log gene copies g^{-1} soil ……**				
Rate										
Control	6.90b†	8.35	7.06	8.66	6.94	6.96c	8.49	7.07b	8.89	7.07
BC-110	6.95ab	8.32	7.00	8.63	6.95	7.16b	8.51	7.20a	8.90	7.07
BC-220	7.02a	8.30	7.02	8.65	6.97	7.29a	8.53	7.15ab	8.90	7.07
Split-110	6.90b	8.30	6.93	8.57	6.93	7.14b	8.47	7.07b	8.93	7.05
Split-220	7.00ab	8.28	7.01	8.64	6.86	7.17b	8.51	7.18a	8.90	7.07
Timing										
Control	6.90b	8.35	7.06	8.66	6.94	6.96c	8.49	7.07b	8.89	7.07
Broadcast	6.95a	8.32	7.03	8.65	6.95	7.14a	8.51	7.14a	8.90	7.07
Split	6.93ab	8.31	7.00	8.62	6.91	7.09b	8.49	7.11ab	8.91	7.06
Time (Sampling event)										
Pre-SD	6.68d	8.39b	6.98bc	8.65b	6.85c	7.04b	8.77a	7.41a	9.02a	7.34a
Post-SD	7.71a	7.91d	6.62d	9.22a	6.60d	7.03b	8.75a	7.43a	8.97a	7.31a
Pre-IRR	6.53e	8.39b	7.22b	8.67b	6.71d	7.26a	8.80a	6.86b	8.83b	7.17b
Post- IRR	6.85c	8.46ab	7.52a	8.50b	7.17a	7.23a	8.45b	6.97b	8.98a	7.05c
Pre-BF	7.06b	8.28c	6.89cd	8.59b	7.26a	6.60c	8.13c	6.61c	8.67c	6.83d
Post-BF	6.74cd	8.54a	6.92c	8.23c	7.01b	7.21a	8.09c	7.37a	8.93ab	6.73e
Rate	*‡	NS	NS	NS	NS	**	NS	*	NS	NS
Timing	NS¶	NS	NS	NS	NS	**	NS	*	NS	NS
Time	**	**	**	**	**	**	**	**	**	**
Rate x Timing x Time	NS	NS	NS	**	NS	**	NS	*	NS	NS

† Different letters within the same column indicate a significant difference between means.

‡ *, **, *** Significant at $p \leq 0.05$, 0.01, and 0.001, respectively.

¶ Not significant.

3.5.6 **Relationships between management practices and soil properties, enzyme activity and gene abundances**

Pearson correlation coefficients were used to compare effects of timing and rates of N application and management events (irrigation and N application) on microbial enzymes activities responsible for N conversion, N cycling functional genes and soil gravimetric moisture content on N_2O emissions (Figs 3.8 and 3.9).

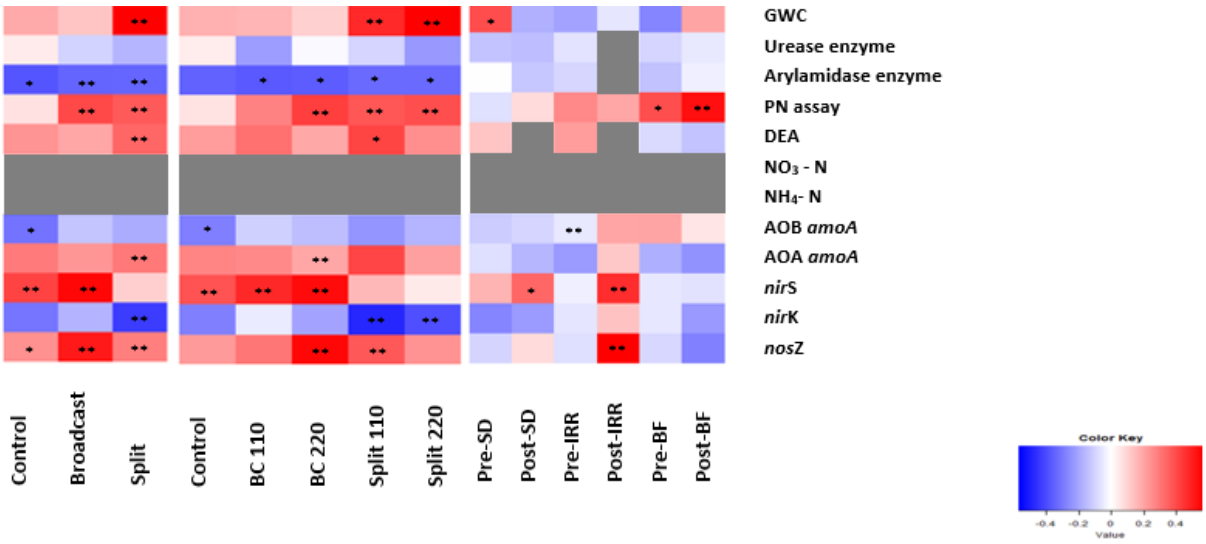

Fig. 3.8 Spearman correlations of N_2O emissions with GWC, enzyme activity, N cycling functional genes, NO_3-N and NH_4-N as affected by rate and timing of N application and sampling event in soil under irrigated canola in 2015. The correlation coefficients ranging from negative to positive are indicated by colour intensity changing from blue to red. *p<0.05, **p<0.01(*p<0.05, **p<0.01, SD – seeding, IRR – irrigation, BF – bolting fertilization and the gray shading indicate no data).

Soil moisture content was positively correlated with N_2O emissions when N was applied as a split application either as Split-110 or Split-220 ($p < 0.01$) (Figs 3.8 and 3.9). In 2015 GWC pos-

itively correlated with N_2O emissions before seeding and in 2016 before and after irrigation. Nitrous oxide emission negatively correlated with arylamidase enzyme activity, across all N treatments only in 2015. Potential nitrification showed a positive correlation with N_2O emissions in both years of the study (Table A.2). In 2015, correlation with N_2O emissions was significant ($p < 0.01$) on BC-220, Split-110 and Split-220; sampling event was significant ($p < 0.01$) after N application at bolting (Fig 3.8). In 2015 positive correlation was observed between DEA and N_2O emissions on Split-110 ($p = 0.05$), while in 2016 DEA and N_2O emissions positively correlated on BC-220 and Split-110 ($p = 0.05$), and Split-220 ($p = 0.01$) (Fig 3.9). Data were not available for NO_3^--N and NH_4^+-N in 2015. In 2016, NO_3^--N was positively correlated with N_2O emissions across all treatments, ($p = 0.01$) (Fig 3.9), NO_3^-N showed positive relationship with N_2O emission after both N and irrigation application. In 2015, AOB *amoA* genes were negatively correlated with N_2O emissions only on control, but positively correlated in 2016 on Split-110 and Split-220 (Fig 3.9). While AOA *amoA* genes, positively correlated with N_2O emissions on BC-220 in 2015 (Fig 3.8). For the correlation analysis of denitrification genes in 2015, *nirS* and N_2O emission showed positive correlation on control, BC-110 and BC-220 ($p = 0.01$). While *nirK* genes show negative correlation with N_2O emissions when N application was Split-110 and Split-220 ($p = 0.01$). *nosZ* gene was positively correlated with N_2O emissions on BC-220, Split-110 and after irrigation event in 2015 (Fig 3.8). However, in 2016, there was no significant relationship between *nirS* gene and N_2O emissions on all N applications. But *nirK* gene corelated positively with N_2O emissions when N was applied as BC-220, Split-110 and Split-220. While positive correlation between *nosZ* genes and N_2O emissions was only observed after irrigation event ($p = 0.05$) in 2016 (Fig 3.9).

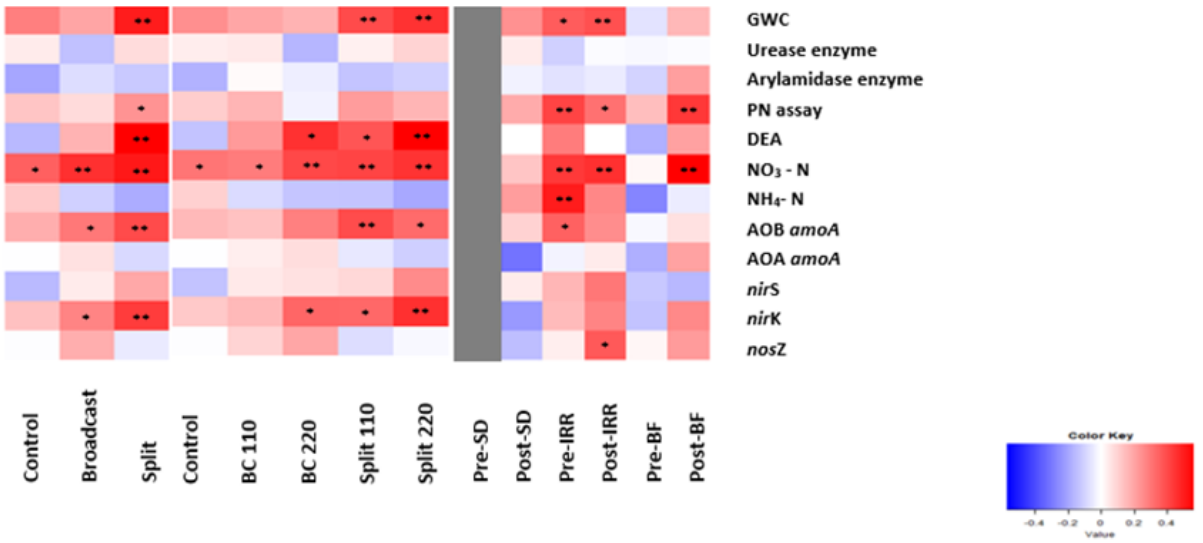

Fig. 3.9 Spearman correlations of N_2O emissions with GWC, enzyme activity, N cycling functional genes, NO_3-N and NH_4-N as affected by rate and timing of N application and sampling event in soil under irrigated canola in 2016. The correlation coefficients ranging from negative to positive are indicated by colour intensity changing from blue to red. (*p<0.05, **p<0.01, SD – seeding, IRR – irrigation, BF – bolting fertilization;gray shading indicates no data).

3.6 Discussion

This is the first study to look at the effect of timing and rate of N application on N_2O emissions, immediately after N application and irrigation, with corresponding changes in microbial abundance and activity, in irrigated canola in semi-arid region in Canada. Most studies look at cumulative emissions over the growing season. Thus, this study can help to understand the immediate contribution of soil microorganisms to N_2O emissions in irrigated canola. Consistent with previous studies with different crops (Gao et al., 2013; Glenn et al., 2012; Wang et al., 2011; McSwiney and Robertson, 2005; Ruser et al., 2001), this study shows that high rates of N_2O emissions shortly after N fertilizer application were always associated with higher soil moisture content or irrigation events. My results show that N_2O emissions increased with increasing rate of N application, as reported in previous studies (Tariq et al., 2017; Zou et al., 2005), irrespective of whether N was broadcast 100% at seeding or 50% at seeding and 50% at bolting. NH_4^+ and NO_3^- availability in the soil, as substrate for nitrification and denitrification processes, would be the driver for N_2O

emission among other factors as moisture, temperature and C availability (Rochette et al., 2014; Gao et al., 2013), and can account for N_2O emission variability in soils. Available soil N data in 2016 shows increased NH_4^+ in the soil after N application in all treatments except the control, with the highest increase on BC-220. N_2O emission was higher in 2015 than 2016 on all N application rates and after management practice of fertilizer and irrigation application. This might be as a result of increased microbial biomass, and consequently increased potential for N_2O emission (particularly from denitrification) as indicated by increased arylamidase activity. Nitrous oxide production in the soil is a result of soil mineral N transformations as determined by soil redox potential, defined by soil moisture content or water-filled pore space (WFPS) (Rochette et al., 2008b; Linn and Doran, 1984). Although emission was higher in 2015 compared to 2016, the highest emission measured was after irrigation representing a 962% and 784% increase in emission compared to the lowest emission measured in 2015 (after seeding) and 2016 (before bolting) respectively (Table 3.3). Environmental conditions can mask the impact of soil mineral N content when soil moisture level favours high redox potential and low denitrification activity (Rochette et al., 2018).

The influence of soil management practices on soil microbial response to varying climatic conditions, soil moisture contents, soil porosity and soil organic matter has been pointed out in previous research works (Chen et al., 2019; Shi et al., 2015). Soil microbial abundance and enzyme activity are both important biological indicators to evaluate soil fertility and N cycling, reflecting the conversion capacity of soil N and other nutrients (Li et al., 2016a). However, soil microbial functions can be impeded by several factors, such as soil water, substrate availability and oxygen availability (Li et al., 2016b; Zhu et al., 2016; Niu et al., 2012). Here, we observed that urease, the hydrolytic enzyme responsible for urea conversion to NH_4^+, decreased after 100% application of urea fertilizer at seeding in 2015. The decrease in urease activity following a single application of N may have been due to a toxic effect of fertilizer on soil microorganisms. For example, high ammonia concentration, osmotic stress and/or soil acidity can be inhibitory at high N fertilizer rates (Du et al., 2018; Campbell et al., 2011; Zhang et al., 2008). In this study, split application of N promoted urease, arylamidase and potential for nitrification in 2015 when moisture limitation was more apparent based on precipitation distribution (Fig 3.2 and 3.3). However in 2016, timing of N application did not affect arylamidase activity. Potential denitrification enzyme activity was

higher in 2016 than 2015 with the split application of N, and this could be as a result of increased soil moisture content prior to the second application of N with irrigation water. Banerjee et al. (2016), showed that soils that were previously wet produced more N_2O than soils that were previously dry, even though they had the same current moisture level. Increased soil water affects soil aeration, which not only affects the growth of soil nitrifiers under already wet soil condition, but also inhibits the growth rate of microorganisms. Reduced O_2 content in the soil directly influenced the synthesis and activity of denitrifying enzymes (Sexstone et al., 2010). Likewise, soil moisture content affects the concentration of fertilizer in the soil water. At low soil moisture, fertilizer concentration may be toxic and affect microbial responses as was possible in this study in 2015. For example, urease enzyme activity decreased immediately after N was applied at the highest rate of 220 kg N ha^{-1}.

Sampling time in this study was to capture microbial response just before and immediately after management practices of fertilizer application and irrigation. This is different from most studies that look at seasonal responses, typically measured over a few weeks. I found that urease enzyme activity tended to decrease immediately after N application, though the decrease was not significant ($p = 0.09$ in 2015 and $p = 0.10$ in 2016). In 2015 (but not 2016), arylamidase enzyme activity ($p < 0.05$) and potential for nitrification were higher after seeding. This suggests that increased microbial biomass, organic and inorganic N mineralization in 2015 under low soil moisture content and potentially higher aeration status may be promoting nitrifier-denitrification. Under well-aerated condition or reduced soil moisture level, availability of NH_4^+ and N_2O formation may be enhanced due to decomposition of inorganic N or reduction of NO_2^- via nitrifier denitrification (Wrage et al., 2004). Irrigation in 2016 decreased arylamidase enzyme activity and potential nitrifiers, possibly as a result of increased soil moisture and reduced O_2 for nitrifier growth, suggesting that balance of water and O_2 availability is required for optimum microbial activity (Moyano et al., 2013). Soil N processes catalyzed by aerobic microbes might be affected by increase in soil water content, Du et al. (2018), concluded that soil O_2 deficiency and excessive N application are not conducive to higher soil microbial activity, which is in agreement with our findings on second application of N on enzyme activity in 2016 when soil moisture condition was higher.

Nitrification is the microbial oxidation of NH_3 to hydroxylamine and subsequent conversion to NO_2^- and NO_3^-. It is the rate limiting step catalyzed by the ammonia monooxygenase enzyme (AMO), which exists both in AOB and AOA (Bothe et al., 2000) and affects plant nutrient availability, nitrate leaching to groundwater, and N_2O release into the atmosphere, with significant consequences for agriculture and the environment (Yao et al., 2011). The abundance of AOA was higher than AOB in all soils, which is in agreement with previous studies and in different ecosystems (Könneke et al., 2005; He et al., 2007; Shen et al., 2008; Wang et al., 2017; Hink et al., 2017). However, AOB has been observed to dominate NH_3 oxidation in microcosms studies supplied with high rates of N fertilizers (Xia et al., 2011; Di et al., 2009;). Other studies also corroborate AOB response to increase N application (Ouyang et al., 2016; Norman and Barrett, 2014), supporting our findings that AOB gene abundance increased with increasing rate of N. In this study, AOB gene abundance increased with N application and was not affected either when 100% of the N was applied at seeding, or when 50% was applied at seeding and 50% at bolting. Likewise, AOA gene abundance was not affected by the rate of N application, similar to other findings that AOA abundance remained stable or even decreased with high rates of N application (Verhamme et al., 2011; Di et al., 2009, 2010). Suggesting that AOB maybe more responsive to change in soil management (Huang et al., 2019) than AOA. Denitrification enzymes are encoded by the functional genes: *nir*S, *nir*K and *nos*Z (Harter et al., 2014). Nitrite oxide reduction to nitric oxide is catalyzed by two structurally different nitrate reductases, one containing heme c and heme d1 (cd1-Nir) encoded by *nirS* gene and the other copper (Cu-Nir) encoded by the *nirK* gene (Zumft, 1997; Wang et al., 2017). In this study I found that in the two sampling years *nirK* and *nosZ* gene abundances were not affected by increasing rate of N application or timing of N application. Rather, denitrification genes responded more generally to soil moisture and nitrate availability. The greater soil moisture and NO_3^- availability at management events created a condition where denitrifier community is primed and ready to produce N_2O on an event driven basis. My result shows that N addition irrespective of timing of application and rate of application did not affect the abundance of denitrification genes, suggesting that N application might only shift denitrifier abundance in the long term (Jin et al., 2014) rather than short period of days (Tatti et al., 2012). In 2016 *nirS* gene copy number increased with a single application of N and split application of N at the highest rate of 220 kg N

ha^{-1}, this might be a response to increased soil moisture content and nitrate concentration, as reported by Enwall et al. (2010). *nos*Z gene abundance in the two study years was driven by management practices rather than rate or timing of N applications. However, the environmental condition seems to be the determinant factor. In 2015, with higher N_2O emissions, *nos*Z genes were upregulated following irrigation, but remained stable or reduced after N application in 2015 and 2016. This is in line with Henry et al. (2006) that N fertilization under continuous corn or corn soybean rotation did not affect the abundance of *nosZ* genes. This suggests denitrification was the dominant process responsible for increased N_2O emissions at these events. In this study, these events occur at split application of N with additional water when irrigation was applied. Soil moisture content is one of the key factors that drive N_2O emissions, as increased soil moisture could result in reduced soil aeration (Gillam et al., 2011). Elevated NO_3^- level in the soil, as a result of N addition and reduced aeration as a result of increase soil moisture content will be favourable to denitrifying enzymes, as denitrification is influenced by management practices such as fertilization, drainage and irrigation (Snyder et al., 2009), through their effects on aeration and availability of NO_3^- (Perron et al., 2019). The availability of NO_3^- can be affected by the rate (Gao et al., 2013; 2017) and timing of N application (Burton et al., 2011; Zebarth et al., 2011), in agreement with the finding from this present study (Fig. 3.5). Under well-aerated condition or reduced soil moisture level, availability of NH_4^+ and N_2O formation may be enhanced due to chemical decomposition of inorganic N or reduction of NO_2^- via nitrifier denitrification (Wrage et al., 2004), and nitrification becomes increasingly effective. In this study in 2015 which is a drier year compared to 2016, mineralization of organic matter as measured by increase in arylamidase enzyme activity, became increasingly important, and nitrification potential was positively correlated with N_2O emission. Temperature can greatly promote soil N_2O emissions when soil moisture or the substrate are not limiting factors (Dobbie and Smith, 2001), thus promoting the decomposition of soil organic matter and improve microbial enzymes activity, causing large emissions as recorded in 2015 (Figures 3.2 and 3.4). In this study, nitrifier-denitrification might be more important in drier 2015 with higher N_2O emissions. High emissions observed after irrigation and when N was applied at bolting, related to water addition, and N application. Irrespective of the rate of N application abundance of *nos*Z genes responsible for N_2O conversion remain stable, while potential for nitrification (Fig. 3.7) and nitrifier gene abundance (Table 3.5) increased with increasing N application rates.

This suggests an increase in NO_3^- availability with increasing rate of N application, while potential for conversion to elemental N remain constant, thus increasing emission with nitrification as the driving process. The condition of our study fields varied across the two growing seasons due to climatic factors such as precipitation and temperature. 2016 being the wetter year, denitrification seems to be the more favorable and dominant N_2O production process as irrigation application and N application further promote denitrification process. In 2016 there was significant positive correlation between N_2O emission and denitrification enzyme activity and increased substrate availability for denitrification with increasing rate of N application.

3.7 Conclusions

Legacy soil conditions before fertilizer and irrigation application plays a significant role in the N_2O production pathway and the magnitude of emission (Banerjee et al., 2016). This study shows that the biological processes driving N_2O production in irrigated canola are more dependent on the prevailing soil condition, water addition via irrigation and N application either applied as 100% at seeding or split application (50% at seeding and 50% at bolting). The increase in emission rates after N application was associated with soil moisture content. Even though N_2O emissions increased following N addition, they were primarily associated with existing moisture conditions prior to further application of water, which was primed with resource availability. This highlights the importance of soil moisture content on N_2O emission; irrigation event planning should take soil moisture condition into consideration. Also, high rate of N application if not proven to be beneficial to yield increase, should be avoided. Nitrogen applied at a high rate, regardless of whether it was applied at once or in split application increased N_2O emission.

4. IDENTIFICATION OF MICROBIAL ACTIVITIES RESPONSIBLE FOR GREATER THAN EXPECTED RESIDUE-INDUCED N_2O EMISSIONS FROM CANOLA

4.1 Preface

Many studies have shown that canola residue instigated higher nitrous oxide (N_2O) emission from soils, either directly from canola or crops following canola in a rotation. Nothing is known about its effect on biological processes governing N_2O emissions from soil. Consequently, this study was conducted using ^{15}N and ^{13}C labelled residues of canola, flax, pea and wheat to follow source of N and effect of residue C on soil microbial community abundance and structure, and relationship with N_2O emissions between residue types.

4.2 Abstract

Oilseed residues, particularly canola (*Brassica napus* L.), instigate higher nitrous oxide (N_2O) emissions compared to pulse and wheat crop residues. Understanding the effect of canola residue on biological processes related to N_2O emission in canola production systems will offer possibilities to increase sustainable production and N use efficiency. To tackle this problem, we conducted an incubation experiment (84 d) using ^{15}N and ^{13}C labelled residues of canola, flax, pea and wheat applied at rates equivalent to those reported for crops grown in the field in Saskatchewan. We used a combination of molecular techniques (qPCR, PLFA and ^{13}C-PLFA) and biogeochemical analysis (DON, DOC, NH_4^+ and NO_3^-) to investigate the N-cycling gene abundance, microbial abundance, community structure, soil biogeochemical properties and quantify N_2O and CO_2 emissions associated and induced by each of the added residues. The magnitude of N_2O emission from residue amended soils was significantly higher ($p < 0.05$) than unamended soil (without residue addition) and differed with residue type: canola > pea = wheat > flax > control. Residue addition significantly increased microbial abundance ($p = 0.001$), denitrification (*nirS*, *nirK*, *nosZ* I and *nosZ* II) but not nitrification (bacteria and archaeal ammonia monooxygenase [*amoA*]) gene abundances, compared to the unamended soil, with canola residue inducing higher denitrifier abundance and higher ^{13}C distribution in copiotrophic microorganisms (G- and fungi). Pearson correlation of DOC and denitrification gene abundance were significant ($p < 0.05$) across all residue treatments. Both canola and pea residue addition resulted in a significant increase in DOC, but only canola residue

resulted in decreased DON and NO_3^-. This suggests an interplay between biologically available C and N that differed among residue types, affecting N_2O emissions. Analysis of $^{15}N_2O$ and $^{13}CO_2$ data shows that the stimulatory effect of canola residues on N_2O emissions is due to differences in microbial assimilation of residue C and the resultant shift in microbial community structure.

4.3 Introduction

Nitrous oxide is a climate-relevant greenhouse and stratospheric ozone depleting gas (Kesenheimer et al., 2019; Yamamoto et al., 2017) with a global warming potential that is 298- and 28-times greater than that of carbon dioxide (CO_2) and methane (CH_4), respectively (Hu et al., 2019). Nitrous oxide emissions from the soil are a result of microbial processes—namely nitrification and denitrification—acting on soil-, fertilizer- and/or crop residue-N (Butterbach-Bahl et al., 2013; Baggs and Philippot, 2010). A recent study by Farrell et al. (2015) showed that there is considerable potential for N_2O emission from decomposing crop residues, and that this is especially true for oilseed (canola and flax) residues. The findings of a recent field study conducted in Saskatchewan also found that N_2O emissions can be higher for wheat grown on canola stubble than on either wheat or pea stubble (Lemke et al., 2018). Likewise, results from a field study comparing canola and chickpea found that cumulative emissions from the canola were 10-times greater than those from chickpea (Schwenke et al., 2010). The authors attributed the differences in N_2O emissions between the two crops to differences in the quality of their residues—though "residue quality" was not defined. It was also reported that although N immobilization and reduced N_2O emissions were linked to the decomposition of low C:N ratio residues (such as canola) during the initial stages of decomposition, canola roots released about twice as much N_2O as the residues of wheat or sorghum during the latter stages of decomposition. Again, this suggests that the composition, or quality, of the residue plays an important role in the transformation of residue- and soil-N to N_2O.

The amount of N_2O emitted depends on several soil factors such as soil aeration and the availability of inorganic N and organic C substrates, all of which are affected by agricultural practices such as N fertilizer application, crop residue management, tillage practice, and cover crop management (Yamamoto et al., 2017; Basche et al., 2014; Rochette, 2008a; Baggs et al., 2006;). The decomposition of crop residues in the soil provides a source of readily available C and N that can

stimulate microbial activity and, in turn, lead to increased N_2O production/emissions (Xia et al., 2014; Shang et al., 2011; Aulakh et al., 2000). As an organic N fertilizer, crop residues are subject to microbial N mineralization and eventual nitrification—during which N_2O is produced (Baggs, 2011). Crop residues also affect denitrification by serving as a C and energy source for denitrifiers, thus enhancing N_2O production under anaerobic conditions (Chen et al., 2013a).

Crop residue additions impact soil physical and chemical properties that, in turn, can influence soil N_2O emissions. Crop residue decomposition may result in the development of anaerobic microsites in the soil by modifying soil aggregation and increasing microbial oxygen (O_2) demand, thus dictating nitrification and denitrification processes (Bakken et al., 2012; Schaufler et al., 2010). The aeration status of the soil—which significantly impacts microbial nitrification and denitrification—is determined largely by the soil water content. In general, water-filled pore space (WFPS) serves as a proxy measure of soil aeration, and is closely related to microbial activity in soils (Linn and Doran, 1984). Chen et al. (2013) reported that soil N_2O emissions are driven by nitrification at 30–60% WFPS, and that denitrification dominates at 50–90% WFPS. However, because of the potential for anaerobicity due to an increase in microbial metabolism associated with increased C inputs from crop residues, denitrification can contribute up to 90% of total soil N_2O emissions at 75% WFPS (Khalil and Baggs, 2005). Returning crop residues to the soil has many beneficial effects, such as nutrient transfer, C sequestration, and erosion control and can improve soil fertility and grain yields (Memon et al., 2018; Turmel et al., 2015; Chen et al., 2013). However, it also impacts the regulation of soil C and N availability and microbial activity that affects N_2O emissions (Gregorutti and Caviglia, 2017; Miller et al., 2008). Opinions from literature reviews differ on the effect of returning residue on N_2O production, with studies demonstrating both a stimulatory (Shang et al., 2011; Mutegi et al., 2010) and inhibitory effect (Sander et al., 2014; Ma et al., 2010) of crop residues on N_2O emissions. Crop residue additions also can influence the ratio of N_2O to N_2 produced; e.g., Li et al. (2013) reported that residue additions reduced soil N_2O production under O_2-limiting conditions and suggested that this was a result of enhanced reduction of N_2O to N_2.

Soil C budgets and nutrient cycling processes are regulated by soil microbes through their influence on decomposition and incorporation of organic material (Fanin et al., 2019; Cotrufo et

al., 2013; Schimel and Schaeffer, 2012). Indeed, understanding how substrate availability influences soil microbial community structure and function is important to determining the fate of C, N, and other nutrients during residue decomposition. The addition of a readily available C source (e.g. crop residue) to soil usually stimulates copiotrophic microbial communities with high growth rate (Fanin et al., 2019; Pascault et al., 2013). This study investigated why canola residues induce greater than expected N_2O emissions. The objectives of the study were to (*i*) use ^{15}N- and ^{13}C-labelled residues of wheat, pea, flax and canola to quantify the source of N_2O and CO_2 emissions; (*ii*) assess changes in the abundance of gene copy number for key steps in the microbial nitrification and denitrification processes; (*iii*) characterize abundance and community structure of residue ^{13}C assimilating microorganisms; and (*iv*) explore relationships between microbial communities, soil chemical properties, and N_2O emissions. Here I hypothesized (i) N_2O emissions following the addition of canola residues are greater than those following the addition of wheat residues, and (ii) N_2O emissions respond to changes in soil N-cycling gene abundance.

4.4 Materials and Methods

4.4.1 Preparation of ^{15}N and ^{13}C labelled residues

Soil was collected from unfertilized research plots at the University of Saskatchewan Goodale Research Farm. The soil had a loam texture, a pH of 7.6, and an organic matter content of 1.7%; soil test results (*ALS Laboratory Services*, Saskatoon) indicated that the soil was low in N (12 kg NO_3-N ha^{-1}), P (16 kg P ha^{-1}) and S (11 kg SO_4-S ha^{-1}), but had adequate K (436 kg K ha^{-1}). To ensure that the added ^{15}N-labeled fertilizer was used efficiently, and to facilitate recovery of the plant roots at harvest, the soil was mixed with sand (60:40 soil:sand, w/w) and approximately 1.6 kg of the soil-sand mixture placed in each of 16 plastic containers (60-cm × 40-cm × 30-cm; l×w×d). Each of the four crops [wheat (AC Barrie), canola (Dekalb 72-65 RR), flax (cv. CDC Bethune), and pea (cv. CDC Treasure)] were then seeded into replicate (n = 4) containers and placed on a tented bench in the greenhouse (Fig. 4.1).

Fig 4.1 ^{15}N and ^{13}C labelling of residue in the greenhouse.

One week after seedling emergence, the plants received 250-mL of a fertilizer solution prepared by dissolving 127.5 g Miracle Grow® All Purpose (24% N) plant food, 68 g K_2SO_4 and 0.69 g ^{15}N-urea (98 atom% ^{15}N) in 8.5 L of distilled water. The fertilizer solution contained an excess (1 atom%) of ^{15}N, and each container received the equivalent of 75 kg N ha^{-1}, 25 kg P_2O_5 ha^{-1}, 115 kg K_2O ha^{-1}, and 30 kg S ha^{-1}. The fertilizer solution was added to the soil between the plant rows (making sure not to get fertilizer solution on the plant leaves) and washed into the soil using additional increments (2 L) of water. Soil moisture was monitored using a Rapitest-mini Soil Moisture Meter (Luster Leaf® Products, Inc.; Woodstock, IL) and was maintained at 75 ± 5% of field capacity throughout the growing season.

The plants were labelled with ^{13}C by introducing an excess of ^{13}C-labeled CO_2 (*Cambridge Isotope Laboratories, Inc.*; Tewksbury, MA) into the atmosphere inside the tent and allowing the plants to take up the $^{13}CO_2$ during photosynthesis. To accomplish this, the tent (1.5-m × 4.3-m × 1.4-m; h×l×w; V = 9.03 m^3) was closed and a known volume (0.2 L to 0.6 L) of $^{13}CO_2$ (99 atom%) injected into the enclosed atmosphere. Fans placed inside the tent were used to ensure that the $^{13}CO_2$ was thoroughly mixed and homogenously distributed throughout the tent, and the amount of CO_2 added was increased as the rate of photosynthesis increased (i.e., as the plants became

larger). The CO_2 concentration in the enclosed atmosphere was monitored using a CAP PPM-3 CO_2 Monitor/Controller and when the CO_2 concentration dropped below the lower set-point (200 ppmV), the tent was opened and remained open until the CO_2 concentration reached ambient levels in the greenhouse (*ca*. 502 ± 70 ppmv); the tent was then closed and the labelling procedure repeated. The labelling procedure was carried out twice per week—beginning two weeks after plant emergence and ending approximately two weeks prior to harvest.

Plants were harvested when they reached maturity (11–15 weeks after emergence depending on the crop) and hand-threshed. The seed was separated from the above-ground biomass and both the seed and remaining straw residue (i.e., leaves + stems + pods) were dried in a forced-air oven at 60°C to a constant weight. Roots were carefully removed from the soil-sand mixture using a 2-mm sieve and tweezers; washed on a 0.5-mm sieve with deionized water; and dried at 60°C to a constant weight. The straw and roots were then coarsely ground using a coffee grinder, and subsampled—with the subsamples being finely ground using a ball mill. The finely ground plant samples were then analyzed using isotope ratio mass spectrometry (IRMS; Delta V Advantage, Isomass Scientific Inc.; Calgary, AB) to determine total N, total C, ^{15}N, and ^{13}C.

4.4.2 **Laboratory incubation**

Soil for the microcosm studies was collected from research plots used in a study examining the impact of N rate, timing, and placement on canola production and greenhouse gas emissions at the Canada-Saskatchewan Irrigation Diversification Centre (CSIDC) in Outlook, SK (*ADF Project No. 20130130*). The soil was transported to the *Prairie Environmental Agronomy Research Laboratory (PEARL)* in the Department of Soil Science at the University of Saskatchewan where it was air dried and screened to pass a 2-mm sieve and remove any visible plant residue, and then sent to *Farmers Edge Laboratories* (Winnipeg, MB) for analysis. The soil had a sandy loam texture, a pH of 7.5, EC of 0.86 dS cm^{-1}, and OM of 2.8%. Soil test results indicated that the soil was high in available N (119 kg NO_3-N ha^{-1}), P (78 kg P ha^{-1}), K (516 kg K ha^{-1}), and S (36 kg SO_4-S ha^{-1}). Total organic C (1.42%) and total N (0.19%) content of the soil (and residues) were determined in the Department of Soil Science, using a standard dry-combustion technique (Skjemstad and Baldock, 2007; McGill et al., 2007).

The treatments included a blank (no soil and no residue; used to obtain $\delta^{15}N$ values for the laboratory air); a control (soil with no residue; used to determine background emissions); and soil amended with one of four crop residues (canola, flax, pea, or wheat). Each treatment combination was replicated four times, with the microcosms arranged in a completely randomized design. An additional set of "destructively sampled" microcosms (n = 96: 6 trt × 4 sampling times × 4 reps) used for microbial analyses (see Section 4.3.4) were included and gas sampled.

Prior to the start of the microcosm study, the soil was brought to (and maintained at) a gravimetric soil water content (GSWC) of 17% (approximately 55% water-holding capacity) to allow the soil microbial community to stabilize—during a three week pre-incubation period at room temperature—and eliminate the CO_2-flux that occurs on rewetting of a dried soil (Lee et al., 2000). At the end of the pre-incubation period, the $^{15}N/^{13}C$-labelled crop residues were incorporated into the soil at rates based on previous studies (Gan et al., 2009) (see Table 4.1).

Table 4.1 Residue additions to the soil microcosms.[†]

Residue		Residue-N		Total N addition		Residue-C		Total C addition		C:N[‡]
(crop)	**amount (mg)**	**(%)**	**(atom% ^{15}N)**	**(mg N)**	**(mg ^{15}N)**	**(%)**	**(atom% ^{13}C)**	**(mg C)**	**(mg ^{13}C)**	
					Above-ground residue (AGR)					
Canola	866.6	1.09	0.6283	9.40	59.06	43.06	1.2729	373.15	4.75	41:1
Flax	541.3	1.74	0.564	9.44	53.24	46.23	1.2577	250.25	3.15	27:1
Pea	675.1	1.10	0.5718	7.45	42.60	42.90	1.2585	289.64	3.65	35:1
Wheat	674.9	0.87	0.5885	5.89	34.66	42.06	1.2333	283.86	3.50	49:1
					Below-ground residue (BGR)					
Canola	219.8	0.86	0.5515	1.90	10.48	42.66	1.1928	93.77	1.12	
Flax	41.20	0.91	0.5147	0.37	1.90	43.05	1.1972	17.74	0.21	
Pea	130.8	1.91	0.5161	2.49	12.85	41.65	1.1681	54.48	0.64	
Wheat	78.80	0.81	0.4993	0.64	3.20	43.91	1.1780	34.60	0.41	

[†] Oven-dry weight of soil added to microcosms = 120 g.

[‡] Above-ground residue + below-ground residue.

All treatment replicates were prepared individually by adding the residue (above- and below-ground residue) to an appropriate amount of moist soil (i.e., equivalent to 120 ± 0.5 g oven-dry weight) in the stainless steel bowl of a Cuisinart® 5.5 Qt. Stand Mixer; the sample was then homogenized by mixing (speed setting = 6) for 2 min. The residue-amended soil was then transferred

into a 40-dram plastic vial, packed to a bulk density of 1.2 g cm^{-3}, wetted with enough water to bring the final water content to 60% water-filled pore space (WFPS), and placed in a 1-L Kilner® jar that was sealed with a polypropylene lid fitted with a self-sealing septum to allow for gas sampling. The jars were then incubated in the dark at room temperature (24 ± 2°C) for 84 d.

4.4.3 **Gas sampling and analysis**

Gas samples were collected throughout the 84-d incubation period, but were sampled with greater frequency at the start of the experiment; i.e., at 4, 18, 24, 36, 48, 97, 145, 193, 242, 290, 362, 483, 604, 727, 971, 1507 and 2025 h after incorporation of the residues. Headspace samples were collected from the microcosms through a sampling port consisting of a gray butyl rubber septum sealed into the lid, and were collected using a 30-cc gas tight syringe. (Note: syringes used to sample the microcosms containing $^{15}N/^{13}C$-labeled residues were not used to sample the blank or control [non-labelled residue] microcosms.) Sampling involved collecting three gas samples from each microcosm: one 20-mL gas sample was collected and transferred to a pre-evacuated 12-mL Exetainer vial (Labco Inc.; Ceredigion, UK) to determine the total concentrations of N_2O and CO_2 using a SCION 456-GC gas chromatograph equipped with a ^{63}Ni electron capture detector (for N_2O) and thermal conductivity detector (for CO_2) (SCION Instruments, Edmonton, AB) (David et al., 2018; Farrell and Elliott, 2007). Two additional 30-mL gas samples were collected and immediately injected into pre-evacuated 22-mL Kimax™ glass vials (Fisher Scientific, Ottawa, ON) for isotopic gas analyses using Picarro isotopic $^{15}N_2O$ and $^{13}CO_2/^{13}CH_4$ analyzers (G-5131-*i* and G-2201-*i*, respectively; Picarro Inc., Santa Clara, CA) (Congreves et al., 2019; Farrell et al., 2019). Nitrous oxide concentrations in the headspace often exceeded the upper limit of the Picarro G5131-*i* analyzer (i.e., 2 ppmv); consequently, samples containing >2 ppmv N_2O (determined using GC) were then diluted with *zero-air* and run on the Picarro G5131-*i* analyzer to obtain the isotopic signature ($\delta^{15}N$) of the N_2O. Dilution effects were accounted for when calculating the total N_2O concentrations. After each sampling event, the microcosms were placed in a fume hood and left open for 20 min to replenish the atmosphere with ambient air. The Kilner® jars were then resealed and returned to the incubator until the next sampling period, when the gas collection/analysis procedure was repeated.

Nitrous oxide production curves were derived by plotting cumulative N_2O-N emissions *vs*. time, and were interpreted using the following response parameters: (i) t_{N2O}, defined as the total cumulative N_2O-N evolved during the incubation; (ii) LAG, defined as the period of time between the initial closure of the microcosms (i.e., at t_0) and the start of the period of rapid N_2O production; (iii) r_{MAX}, defined as the slope of the linear least-squares regression line plotted between the end of the lag period and the start of the plateau region of the cumulative N_2O production curve (i.e., the point at which the R^2 for the least-squares regression line drops below 0.95); and (iv) t_{MAX}, defined as the time (h) required for N_2O production to plateau. The same response parameters were used to describe the cumulative CO_2 production curves.

Residue-derived emissions; i.e., N_2O-N or CO_2-C derived directly from the crop residues, were calculated using Equations 4.1 and 4.2.

$$EF_r = \frac{^{15}N_r - ^{15}N_c}{^{15}N_a} \quad (4.1)$$

$$RDE = (N_r - N_c) \times EF_r \quad (4.2)$$

where EF_r = emission factor for residue-derived N_2O; $^{15}N_r$ and $^{15}N_c$ are the cumulative amounts of N_2O-^{15}N (μg ^{15}N kg^{-1} soil) produced by the residue-amended and control (unamended) soils, respectively; $^{15}N_a$ is the total amount of ^{15}N added as residue; *RDE* = total residue-derived emissions (μg ^{15}N kg^{-1} soil); and N_r and N_c are the cumulative amounts of total N_2O-N (μg N kg^{-1} soil) produced by the residue-amended and control (unamended) soils, respectively. The emission factors and total residue-derived CO_2 emissions were calculated by substituting CO_2-^{13}C and total CO_2-C for N_2O-^{15}N and total N_2O-N in above equations. Residue-induced soil emissions (*RISE*) are defined as the difference between total net emissions (N_{net} or C_{net}) and RDE (Equation 4.3):

$$RISE = N_{net} - RDE \quad (4.3)$$

4.4.4 **Soil microbial abundance and functional group analysis**

DNA extraction and polymerase chain reaction (qPCR). Once gas sampling was completed for a given date, microbial community analyses were conducted using randomly selected microcosms that were destructively sampled at 0, 48, 97 and 362 h after gas samples were collected. Soils

sampled were stored at -80°C until DNA extraction.

DNA was extracted from 0.25g of soil using DNeasy® PowerSoil® Pro kit (QIAGEN, Strasse, Germany) according to the manufacturer's instruction. DNA extracts were stored at -80°C (Smith et al., 2010). Prior to qPCR of archaeal and bacterial *amoA*, *nirS*, *nirK*, *nosZ* clade I and *nosZ* clade II measurement, genomic DNA template concentration of the samples were adjusted to 10 ng μL^{-1} after measurement with Qubit®2.0 Fluorometer using Qubit® dsDNA HS assay kit (Thermo Fisher Scientific, Waltham, MA USA).

The qPCR products for archaeal and bacterial *amoA*, *nirS*, *nirK*, *nosZ* clade I and *nosZ* clade II were amplified with an ABI StepOnePlus Real-time PCR system (Life Technologies, Burlington, ON) using SYBR green detection chemistry. Standards were prepared using an Invitrogen TOPO TA Cloning Kit (Life Technologies, Carlsbad, CA) to insert amplified target templates into the vector, the primer pairs for the desired targets were selected based on literature as indicated in appendix A Table A1. Colonies successfully cloned with the insert (white coloration) were cultured using liquid broth, following the protocol in the TOPO TA Cloning® kit, incubated at 37°C for 16 h. Qiagen QIAprep Spin Miniprep kit (Qiagen, Mississauga, ON) was used to extract DNA (plasmid with insert) from the culture, the plasmid linearization was done using Hind III restriction enzyme (Life Technologies, Burlington, ON) and run on a 1% agarose gel. The bands with linearized products were excised and the gel purified. A Qubit® 2.0 Fluorometer with Qubit® dsDNA HS assay kit (Thermo Fisher Scientific, Waltham, MA USA) was used to quantified DNA for the gel purification. A 10-fold dilution standard ranging between 10^2 and 10^9 gene copies were prepared with appropriate primers to target key genes in the nitrification (archaeal and bacterial *amoA*) (Liesack et al., 1997; Stephen et al., 1999) and denitrification pathways (*nirS*, *nirK* , *nosZ* clade I and *nosZ* clade II) (Throbäck et al., 2004; Jones et al., 2013). Gene quantification was done using an ABI StepOnePlus Real-time PCR system (Life Technologies, Burlington, ON) and quantification of each sample performed in triplicate in 96 well plates. Detailed description of reaction mixtures and thermocycler conditions can be found in appendix A A1.

Phospholipid fatty acids analysis. Phospholipid fatty acid analysis (PLFA) was conducted according to the method adopted by Helgason et al. (2010) from White et al. (1979) based on the original

method of Bligh and Dyer (1959). ^{13}C-PLFA stable isotope probing was used to determine differences in the microbial abundance and community structures of organisms that assimilated the residue-derived ^{13}C present in different residue-amended soils. ^{13}C natural abundance of microbial biomass was determined using the control. For PLFA analysis, soil samples were sieved, freeze-dried and fatty acids extracted from 4.0 g of lyophilized soil in a methanol/chloroform/phosphate buffer mixture and then dried under constant N_2 flow. Neutral, glyco- and phospho-lipids were separated using solid phase extraction columns (0.50g Si; Varian Inc. Mississauga, ON), sequentially eluted with chloroform ($CHCl_3$), acetone [$(CH_3)_2CO$] and methanol (MeOH) respectively, and the phospholipid fraction dried under N_2 flow. With a solution of 1:1 methanol/toluene and methanolic potassium hydroxide (KOH) at 35°C, phospholipids were methylated. The resulting fatty acid methyl esters (FAMEs) were analyzed using a Scion 436-GC gas chromatograph with an Agilent 25m ultra 2 capillary column (J&W Scientific), and a Flame Ionization Detector (FID), using hydrogen UHP carrier gas. Peaks were identified using fatty acid standards and custom software and quantified based on the addition of a known concentration of the internal standard methyl nonadecanoate (19:0) (Drenovsky et al., 2004; Helgason et al., 2010a).

Total biomass was determined by addition of all named peaks calculated based on the peak area detected for each fatty acid, relative to that of a known quantity of the internal standard. Bacterial biomass was determined using thirteen biomarkers (i14:0, i15:0, a15:0, i16:0, 16:1v7c, 10Me16:0, i17:0, a17:0, cy17:0, 10Me17:0, 18:1v7c, 10Me18:0, and cy19:0), which are further separated to represent gram-positive bacteria (G+) (i14:0, i15:0, a15:0, i16:0, i17:0, a17:0) and gram-negative bacteria (G-), (16:1 ω7t, 16:1ω9c, 16:1ω7c, 18:1ω7c, 18:1ω9c, cy17:0, and cy19:0) (Macdonald et al., 2004). Fungal biomass was assessed using 18:2ω6, 9c (Baath and Anderson, 2003), and actinobacteria assessed using fatty acid 10Me16:0 and 10Me18:0. Physiological stress biomarkers were reported as the ratio of cy17:0 to 16:1ω7c (Stress 1) and cy19:0 to 18:1ω7c (Stress 2) (Arcand et al., 2016). All biomass values are reported based on dry soil weight in units of nmol g^{-1} soil (Helgason et al., 2010a). The C isotope ratio of fatty acids was determined by GC-C-IRMS. DELTA^{PLUS} isotope ratio mass spectrometer (Thermo Fisher Scientific, Waltham, MA) coupled with a Hewlett Packard 6890 series II GC (Agilent, Palo Alto, CA) through a GC Combustion-III interface (Thermo Fisher Scientific, Waltham, MA). Reference CO_2 of known isotopic composition was injected at the beginning of each sample run and a standard mixture of eight fatty acids

of known isotopic composition was run with every analysis period. ^{13}C enrichment of the fatty acid was corrected for the C atom derived from methanol (-43.96‰) during methylation. Total PLFA analysis by Scion 436-GC using custom software identified 60 fatty acid methyl esters (FAMEs). Inadequate separation of some FAMEs during GC-C-IRMS analysis as a result of blending $\delta^{13}C$ signals resulted in identification of isotopic ratios for 31, 40, 33 and 41 FAMEs at 0, 48, 97 and 362 hours destructive sampling respectively, thus necessitating exclusion of the blended FAMEs in our analysis, following steps described by Helgason et al. (2014). Also, the quantity and distribution of ^{13}C incorporated into individual PLFA ($^{13}C_{inc}$) were calculated as described by Helgason et al. (2014):

$$^{13}C_{inc} = (F_R - F_C)*PLFA_s \quad (4.4)$$

Where $PLFA_s$ is the amount (µg g^{-1} soil) of an individual PLFA (^{13}C + ^{12}C) from the residue amended soil as determined by GC-FID, F_C is the fraction of ^{13}C in an individual PLFA from unamended soil and F_R is from ^{13}C residue amended soil and calculated as:

$$F = R/(R + 1) = {}^{13}C/({}^{13}C + {}^{12}C) \quad (4.5)$$

The carbon isotope ratio (R) was derived using GC-C-IRMS measurement, relative to VPDB (0.0112) standard as follows:

$$R = (\delta^{13}C/1000 + 1)*R_{VPDB} \quad (4.6)$$

The percentage distribution of incorporated ^{13}C in an individual fatty acid or biomarker functional groups (% $^{13}C_{M\text{-}DIST.}$) was calculated by dividing $^{13}C_{inc}$ (µg g^{-1} soil) defined as ^{13}C-PLFA in an individual (18:2ω6,9c) lipid or group of lipids (e.g., G- bacteria) by the total amount of $^{13}C_{inc}$ (µg g^{-1} soil) defined as $\sum^{13}C$-PLFA in all identified FAMEs at different destructive sampling period (Moore-Kucera and Dick, 2008) .

$$\%^{13}C_{M\text{-}DIST} = ({}^{13}C\text{-}PLFA/\sum{}^{13}C\text{-}PLFA)*100 \quad (4.7)$$

4.4.5 **Characterization of nutrient content and soil properties**

Water-soluble organic C and N were measured at all destructive sampling times. Water extractable organic matter was extracted from 5 g of soil using 5 mM $CaCl_2$ solution (1:2 w/v)

(Zsolnay, 1996, 2003; Kalbitz et al., 2003), and extract filtered with 0.4 µm polycarbonate filter using a glass vacuum filter unit. The concentration of total dissolved organic nitrogen (DON) and total dissolved organic carbon (DOC) was measured using combustion/non-dispersive infrared gas analysis method by using a total organic C analyzer (TOC-5000A, SHIMADZU). Exchangeable NO_3^- and NH_4^+ were extracted from 5 g of soil by shaking in 50 mL of 2.0 *M* KCl (ratio 1:10). Nitrate-N in the extract was determined using segmented flow analysis (cadmium reduction procedure) and determination of NH_4-N in the extract by segmented flow autoanalyzer (Technicon Instrument Corporation) indophenol blue procedure (Phenate method). Soil gravimetric moisture content was determined by placing 10 g of fresh soil in a conventional oven for 24 h at 105°C (Carter and Gregorich.,2008).

4.4.6 **Statistical analysis**

The software package IBM SPSS statistics v.26 was used to perform a linear mixed model analysis for repeated measures to determine significance of the differences between labelled residue applications for WSOC and N, N_2O emissions, ^{15}N-N_2O emissions, CO_2 emissions, ^{13}C-CO_2 emissions and N functional gene abundances at $p < 0.05$. Least-significant difference (LSD) was used for post-hoc comparison of individual treatments. Pearson correlation coefficients were used to determine the relationship between biogeochemical properties of incubated soils with N cycling functional genes, microbial abundance, N_2O, $^{15}N_2O$, CO_2 and $^{13}CO_2$ emissions as affected by residue application. Principal coordinate analysis (PCoA) using PCOrd v.6 (MjM Software Gleneden Beach, OR) was used to analyze the community composition of the PLFA data and ^{13}C-PLFA % distribution. The Sørensen distance measure was selected in the Autopilot Slow and Thorough analysis option in PCOrd v.6 (McCune and Grace, 2002; Helgason et al., 2010b). A random starting point was used for initial analysis and then optimized in previous ordinations to achieve the lowest stress. Multi-Response Permutation Procedure (MRPP) was subsequently used to test for differences between groups. Correlations between PLFA community structure and other parameters were conducted using a minimum r = 0.7. Phospholipid data expressed as mol% were transformed using log (mol% + 1) for statistical analysis but are presented as mol%.

4.5 Results

4.5.1 Total N_2O and ^{15}N-N_2O emissions

The majority of N_2O emitted from residue-amended soils occurred during the first 290 hours of incubation (Fig. 4.2A). Nitrous oxide emissions from the unamended control soil were quite small and, after a short (4 h) lag period, increased in a near-linear fashion throughout the remainder of the incubation—though at a very low rate (0.01 μg N_2O-N h^{-1}). The residue-amended soils produced considerably more N_2O, with production rates (r_{max}) that were significantly greater than that for the control (Table 4.2). In general, N_2O production from the residue-amended soils was greatest for canola, intermediate for pea and wheat, and lowest for flax—with both the rate and duration of maximum N_2O evolution increasing in the order: flax < wheat ≈ pea << canola. The N_2O production curves for the residue-amended soils essentially plateaued (i.e., exhibited near-zero rates of increase) after *ca*. 700 h, and total cumulative N_2O production at the end of the 84-d incubation increased in the order: control << flax < wheat < pea << canola (Table 4.2).

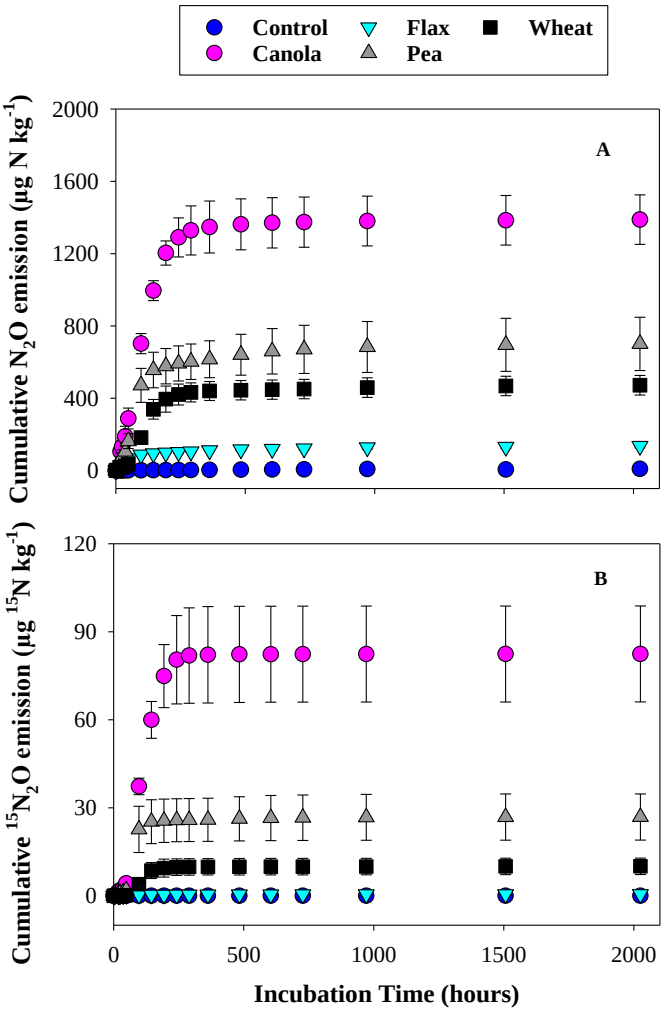

Fig 4.2 Effect of canola, flax, pea and wheat residue addition on (A) cumulative N_2O emission and (B) cumulative $^{15}N_2O$ emission. Error bars indicate standard error of the mean (n=4)

Table 4.2 Total and residue-derived N_2O production during an 84 day incubation at 60% water filled pore space (n = 4).

Crop residue	Total N_2O-N†					Total ^{15}N-N_2O†				
	Lag‡b (h)	Plateau	r_{max}¶ (µg N h^{-1})	t_{max}§ (h)	Cum. N_2O (µg N kg^{-1} soil)	Lag (h)	Plateau	r_{max} (µg N h^{-1})	t_{max} (h)	Cum. ^{15}N-N_2O (µg N kg^{-1} soil)
Control	4	no	0.008 c	971 a	9 d	0	no	0.0003 d	971a	0.03 d
Canola	0	yes	5.12 a	290 b	1388 a	18	yes	0.3456 a	290 b	82.43 a
Flax	0	yes	0.86 c	97 c	135 c	0	yes	0.0024 c	145 c	0.54 c
Pea	0	yes	3.42 b	193 c	701 b	18	yes	0.2364 a	145 c	26.86 b
Wheat	0	yes	1.79 bc	290 b	472 b	18	yes	0.0635 b	193 c	10.05 b

† Within columns, means followed by the same letter are not significantly different at $p \leq 0.05$.

‡ Lag = the period of time between the initial closure of the microcosms (i.e., at t_0) and the start of the period of rapid N_2O production.

¶ r_{max} = the slope of the linear least-squares regression line plotted between the end of the lag period and the start of the plateau region of the cumulative N_2O production curve (i.e., the point at which the R^2 for the least-squares regression line drops below 0.95).

§ t_{max}, defined as the time (h) required for N_2O production to plateau.

Residue-derived N_2O was tracked by determining the amount of $^{15}N_2O$ emitted during the incubation (Fig. 4.2B). In general, $^{15}N_2O$ production followed patterns similar to those for total N_2O (Fig. 4.2A); however, whereas there was no apparent lag period for total N_2O production, lag periods of 1–2 d were observed for $^{15}N_2O$ production from the canola, pea, and wheat residues (Table 4.2). Conversely, there was no apparent lag period for $^{15}N_2O$ production from the flax residue (Table 4.2), though the rate and cumulative amount of N_2O-^{15}N produced were about two orders of magnitude lower than those for the other residues. Emissions of $^{15}N_2O$ from the residues essentially ceased after *ca*. 300 h (Fig. 4.2B). Total cumulative N_2O-^{15}N differed among the residues ($p < 0.05$), increasing in the order: flax < wheat ≈ pea < canola (Fig. 4.2B, Table 4.2).

Direct residue-derived N_2O accounted for 0.1% to 14.2% of the total N_2O produced from the residue-amended soils (Table 4.3), with residue-derived emissions (RDE) increasing in the order: flax < wheat ≈ pea < canola ($p < 0.05$). Residue-induced soil emissions (RISE)—defined as the increase in total N_2O relative to the unamended (control) minus the RDE—accounted for the vast majority (i.e., >86%) of the total N_2O. Although canola was characterized by the lowest %RISE, it induced 2- to 3-times more total N_2O (expressed as μg N kg^{-1} soil) than the pea or wheat residues, and *ca*. 11-times more N_2O than the flax residue (Table 4.3).

Table 4.3 Residue-derived and residue-induced N_2O emissions.

Crop residue	Residue ^{15}N added (μg ^{15}N kg^{-1} soil)	Cum. N_2O-^{15}N (μg N kg^{-1} soil)	EF_r[†]	RDE[‡] (μg N kg^{-1} soil)	RISE[¶] (μg N kg^{-1} soil)
Control	---	0.03 d[§]	---	---	---
Canola	579.5	82.43 a	0.142 a	195.8 a	1183 a
Flax	459.5	0.54 c	0.001 c	0.1 c	126 c
Pea	462.1	26.86 b	0.058 b	40.1 b	652 b
Wheat	315.5	10.05 b	0.032 b	14.8 b	448 b

[†] Emission factor for N_2O-^{15}N produced during the 84-d incubation; expressed as a fraction of the ^{15}N added as residue.

[‡] Total residue-derived emissions.

[¶] Residue-induced soil emissions.

[§] Within columns, means followed by the same letter are not significantly different at $P \leq 0.05$.

4.5.2 CO_2 and ^{13}C-CO_2 emissions

Emission patterns for total CO_2 and $^{13}CO_2$ were similar (Fig. 4.3A and 4.3B, respectively) and, unlike N_2O production, did not plateau during the 84-d incubation period. Total CO_2 emissions from the control (unamended) soil were relatively small and increased in a near-linear (R^2 = 0.984) fashion throughout the 84-d incubation. On the other hand, the residue-amended soils produced CO_2 at much faster rates, and yielded total cumulative emissions that were 3- to 4.5-times greater than those from the control soil (Table 4.4). Total CO_2 emissions from the residue-amended soils increased in the order: flax < wheat < pea < canola. Unlike N_2O emissions, CO_2 emissions continued to increase throughout the 84-d incubation, though the rate of increase had slowed considerably by day 42 (i.e., 1000 h; Fig. 4.3A).

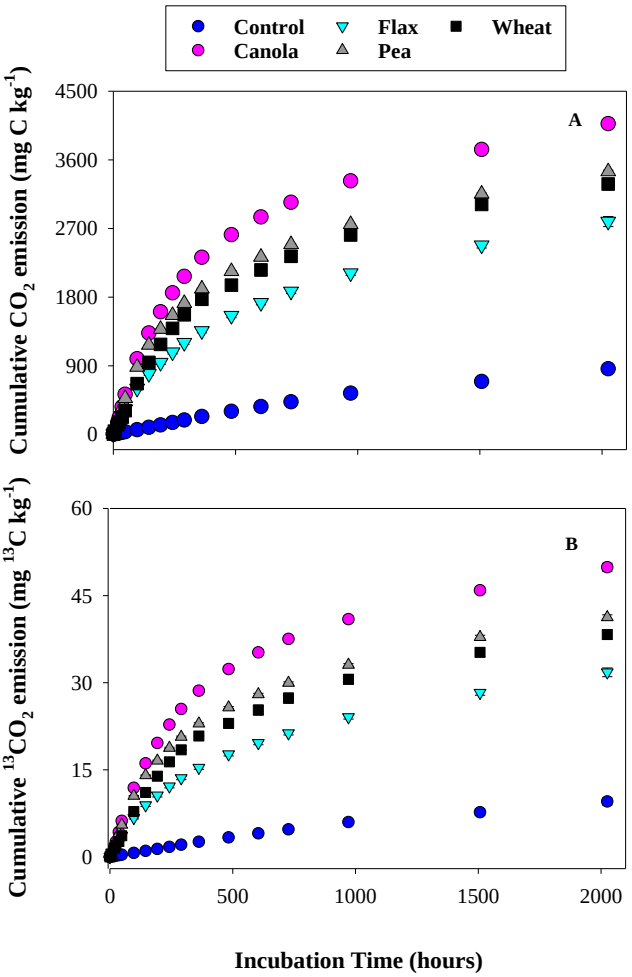

Fig 4.3 Effect of canola, flax, pea and wheat residue addition on (A) cumulative CO_2 emission and (B) cumulative $^{13}CO_2$ emission. Error bars indicate standard error of the mean (n=4).

Table 4.4 Total and residue-derived CO_2 production during an 84 day incubation at 60% water filled pore space (n = 4)

Crop residue	Total CO_2-C †					Total CO_2-^{13}C †				
	Lag‡ (h)	Plateau	r_{max}¶ (mg C h^{-1})	t_{max}§ (h)	Cum. CO_2 (mg C kg^{-1}soil)	Lag (h)	Plateau	r_{max} (mg C h^{-1})	t_{max} (h)	Cum. CO_2-^{13}C (mg C kg^{-1}soil)
Control	0	no	0.47 d	2025 a	917 d	18	no	0.0046 c	2025 a	9.5 d
Canola	0	no	6.76 a	362 c	4075 a	47	no	0.0591 a	483 b	49.9 a
Flax	0	no	3.88 c	362 c	2792 c	36	no	0.0315 b	483 b	31.8 c
Pea	0	no	5.52 a	362 c	3447 b	18	no	0.0611 a	362 c	41.3 b
Wheat	0	no	4.46 b	483 b	3284 b	18	no	0.0585 a	362 d	38.3 b

† Within columns, means followed by the same letter are not significantly different at $p \leq 0.05$.

‡ Lag = the period of time between the initial closure of the microcosms (i.e., at t_0) and the start of the period of rapid CO_2 production.

¶ r_{max} = the slope of the linear least-squares regression line plotted between the end of the lag period and the start of the plateau region of the cumulative CO_2 production curve (i.e., the uppermost point at which the R^2 for the least-squares regression line remains > 0.95).

§ t_{max}, defined as the time (h) required for CO_2 production to plateau.

The amounts of CO_2-^{13}C derived directly from the residues were determined using stable isotope (^{13}C) labeling of the crop residues. In general, and despite a short (18 to 47 h) lag period, the $^{13}CO_2$ production curves (Fig. 4.3B) followed the same general patterns as total CO_2 production (Fig. 4.3A). That is, $^{13}CO_2$ production did not plateau during the 84-d incubation, and cumulative CO_2-^{13}C increased as the amount of ^{13}C added as residue increased; i.e., in the order: flax < wheat ≈ pea < canola (Table 4.4). Emission factors for the residues (EF_r) averaged about 0.85 ± 0.05 (Table 4.5), but were greatest for the pea and wheat residues, and lowest for the oilseed residues.

Table 4.5 Residue-derived and residue-induced CO_2 emissions.

Crop residue	Residue ^{13}C added (mg ^{13}C kg^{-1} soil)	Cum. CO_2-^{13}C (mg C kg^{-1} soil)	EF_r†	RDE‡ (mg C kg^{-1} soil)	RISE¶ (mg C kg^{-1} soil)
Control	---	9.5 d	---	---	---
Canola	48.9	49.9 a	0.83	2609	549
Flax	28.0	31.8 c	0.80	1492	383
Pea	35.8	41.3 b	0.89	2247	283
Wheat	32.6	38.3 b	0.83	2090	277

† Emission factor for CO_2-^{13}C produced during the 84-d incubation; expressed as a fraction of the ^{13}C added as residue.

‡ Total residue-derived emissions.

¶ Residue-induced emissions.

§ Within columns, means followed by the same letter are not significantly different at $P \leq 0.05$.

4.5.3 **Biogeochemical properties of incubated soils**

Soil NH_4^+-N and NO_3-N. Nitrogen (i.e., NO_3^- and NH_4^+) availability exhibited significant residue, time, and residue × time interaction effects (Table 4.6). Initial NO_3^- concentrations ranged from 140 to 165 µg NO_3^- g^{-1} soil, and was greatest in soil amended with pea residue and lowest in soil amended with flax residue and the unamended control. Nitrate concentrations decreased rapidly during the first 48 to 97 h of the incubation ($p < 0.05$) before stabilizing; i.e., there were only small changes in NO_3^- concentration between 97 and 362 h (Fig. 4.4A).

Initial soil NH_4^+ concentrations also increased as a result of the addition of the crop residues (Fig. 4.4B). Unlike NO_3^-, however, the greatest increase occurred following the addition of flax

residue. Regardless, soil NH_4^+ concentrations were relatively low throughout the incubation, ranging from 0.60–4.86 µg NH_4^+ g^{-1} soil. Peak NH_4^+ concentrations occurred at 48 h for the canola residue (3.50 µg NH_4^+ g^{-1} soil), and at 97 h for the pea (4.39 µg NH_4^+ g^{-1} soil) and wheat (4.07 µg NH_4^+ g^{-1} soil) residues. Thereafter, NH_4^+ concentrations decreased and, by the end of the incubation, had returned to background levels.

Table 4.6 Repeated measures analysis of variance (ANOVA) of labelled residue application effect on biogeochemical properties of incubated soils (n=16).

Crop residues	DOC	DON	DOC : DON	NO_3-N	NH_4-N
Control	0.017 d†	0.113 a	0.154 d	115.15 b	2.49 b
Canola	0.024 ab	0.090 c	0.266 ab	102.38 c	2.06 b
Flax	0.021 c	0.105 b	0.206 c	113.30 bc	3.56 a
Pea	0.025 a	0.105 b	0.239 b	117.62 a	2.56 b
Wheat	0.023 b	0.091 c	0.267 a	106.53 bc	2.44 b
Incubation Time					
0	0.026 a	0.093 b	0.274 a	148.86 a	1.78 b
48	0.022 b	0.126 a	0.180 c	111.52 b	3.90 a
97	0.020 c	0.093 b	0.222 b	95.47 c	3.91 a
362	0.020 bc	0.091 b	0.230 b	88.15 c	0.90 a
Crop residues	***‡	***	***	**	**
Incubation Time	***	**	***	***	***
Crop residues x Incubation Time	NS	**	**	*	**

† Different letters within the same column indicate a significant difference between means.
‡ *, **, *** Significant at $p \leq 0.05$, 0.01, and 0.001, respectively.
¶ Not significant.

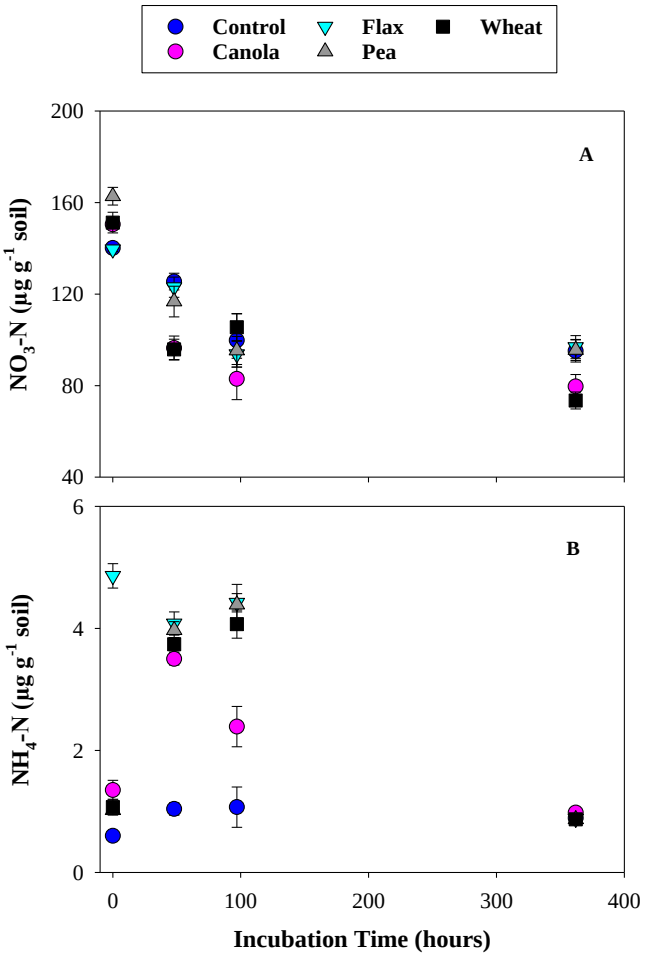

Fig 4.4 Effect of canola, flax, pea and wheat residue addition on (A) NO_3^--N and (B) NH_4^+-N. Error bars indicate standard error of the mean (n=4).

Soil DOC (Dissolved organic carbon) and DON (Dissolved organic nitrogen). Dissolved organic carbon concentrations in the soils increased significantly ($p < 0.001$) upon addition of the crop residues (Fig. 4.5A)—with the greatest increase following the addition of canola and pea residues and lowest following the addition of flax residue. However, DOC concentrations decreased rapidly during first 97 h of the incubation ($p < 0.05$), with little change thereafter (Fig. 4.5A). Whereas residue addition had only a small effect on the initial DON content of the soil, DON concentrations increased in the control and residue-amended soils during the first 48 h (Fig. 4.5B). Thereafter, concentrations of DON decreased sharply over the next 48 h—with the largest decreases occurring in the soil amended with canola and wheat residues. The DOC:DON ratio was greater in the residue-amended soil than the control soil at all sampling times. In general, however, the soil amended with flax residue exhibited the lowest DOC:DON compared to the other residues (Fig. 4.5C).

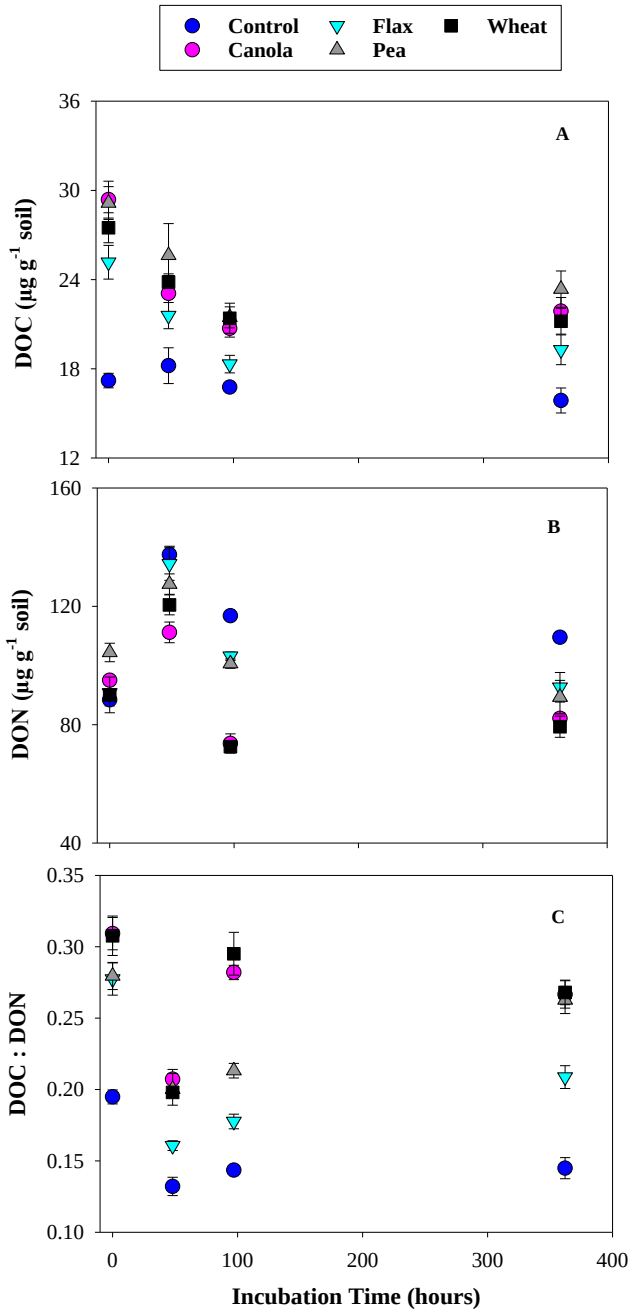

Fig 4.5 Effect of canola, flax, pea and wheat residue addition on (A) DOC (B) DON (C) DOC:DON. Error bars indicate standard error of the mean (n=4).

4.5.4 **Soil microbial abundance and community structure**

Abundance of N-cycling functional genes. For the AOA, *amoA* gene copy numbers (GCN) ranged from 9.07 to 9.75 logGCN g^{-1} dry soil and were about one to two orders of magnitude greater than

those for the AOB, which ranged from 7.73 to 8.32 logGCN g^{-1} dry soil (Fig. 4.6). Residue additions to the soil had no significant effect on the gene copy abundance of either AOA *amoA* (p = 0.51) or AOB *amoA* (p = 0.25). However, relative to the initial value, the abundance of both AOA *amoA* and AOB *amoA* ($p < 0.001$) increased significantly ($p < 0.001$) during the incubation (Fig 4.6), with the gene copy increased at 48 h, slightly decreased at 97 h and becoming stable thereafter.

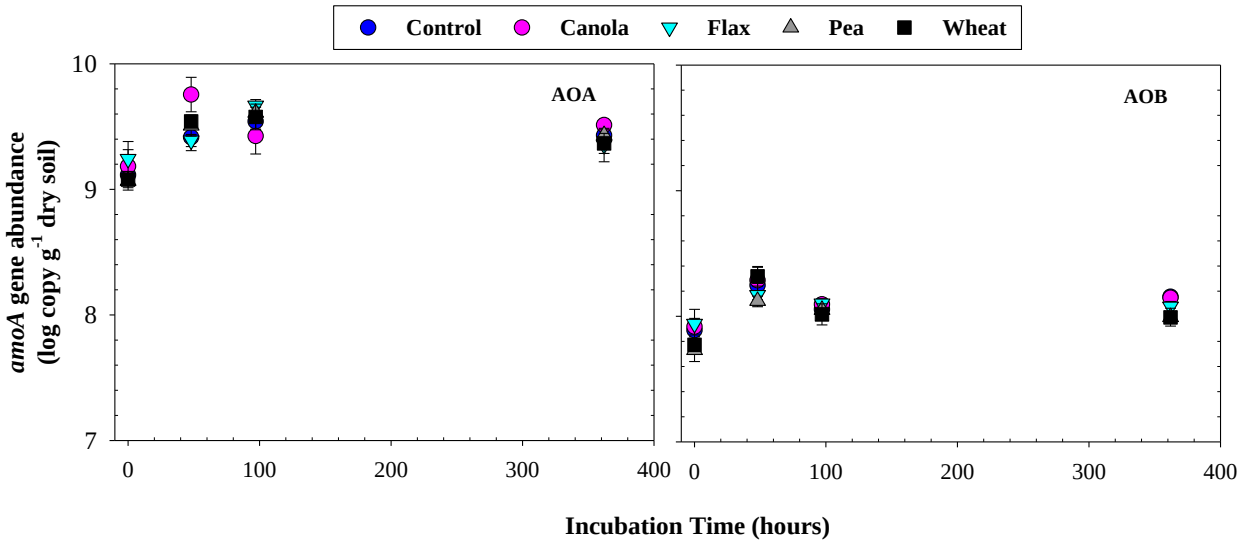

Fig 4.6 Effect of canola, flax, pea and wheat residue addition on *amoA* gene copy abundance. Error bars indicate standard error of the mean (n=4).

The abundance of the *nirS* and *nirK* genes ranged from 6.66 to 8.50 logGCN g^{-1} dry soil and 7.38 to 8.72 logGCN g^{-1} dry soil, respectively (Fig. 4.7). Crop residue addition and incubation time significantly increased *nirS* and *nirK* gene copy abundance (Table B1, appendix), though there was no significant residue × time interaction (Table 4.7). At the start of the incubation, both the unamended control soil and the residue-amended soils had similar *nirS* gene copy numbers and, in all cases, the gene copy numbers increased initially—peaking at 97 h—before decreasing (Fig. 4.7B). Nevertheless, *nirS* gene copy numbers at 362 h remained elevated compared to the time-zero values. The gene abundance pattern for *nirK* differed from that of *nirS* in that it peaked at 48 h, decreased significantly between 48 and 97 h, and then increased between 97 and 362 (Fig. 4.7A). In all soils, the ratio of *nirS* to *nirK* was greatest at 97 h but was otherwise unchanged during the incubation period, between residue types and unamended control (Fig. 4.7C, Table B2).

Gene copy numbers for *nosZ* II were greater than those for *nosZ* I (Fig. 4.8)—with gene abundance for *nosZ* II ranging from 8.32 to 9.07 logGCN g^{-1} dry soil, and for *nosZ* I from 7.43 to 8.62 logGCN g^{-1} dry soil (Fig. 4.8). There was a significant effect of crop residue addition, but no significant interaction between residue addition and incubation time (Table 4.7). Relative to the control soil, *nosZ* I gene abundance was higher in the residue-amended soils ($p < 0.005$) at all sampling times (Table 4.7). *nosZ* II gene copies were not significantly different between residue types and unamended control during between 0 and 97 h and then increased slightly by 362 hours with significant different between control and only canola and pea residues amended soils (Fig. 4.8B). At 48 hours into the incubation, *nosZ* I gene was highest on canola residue between residue types ($p < 0.05$), while between 0 and 97 h *nosZ* II gene copies were not significantly different between the control and residue amended soils (Fig. 4.8). Nitrous oxide reduction potential was assessed by the ratio of *nosZ* I and *nosZ* II gene, higher potential was observed on flax at 0 h, while at 48 h conversion potential was higher for canola and wheat and at 362 h for canola compared to control. At 97 h the potential was not significantly different between residue types and unamended control (Table B1, appendix). The ratio of (*nirS* + *nirK*): *nosZ* I and (*nirS* + *nirK*): *nosZ* II (Fig. 4.9) was calculated as an indicator of N_2O production *vs*. consumption potential. Lower (*nirS* + *nirK*):*nosZ* I was observed on canola at 48 and 97 h (Fig. 4.8A, Table B1) and there was no observable difference between residue-amended and unamended control on (*nirS* + *nirK*): *nosZ* II (Fig. 4.9B, Table B1).

Table 4.7 Repeated measures analysis of variance (ANOVA) and effects of labelled residues of canola, flax, pea and wheat application on N cycling genes in incubated soil at four different sampling times (n=4).

Crop residues	**AOA**	**AOB**	***nirS***	***nirK***	***nosZ* I**	***nosZ* II**	***nirS* : *nirK***	***nosZ* I : *nosZ* II**	**(*nirS*+ *nirK*) : *nosZ* I**	**(*nirS*+ *nirK*) : *nosZ* II**
Control	9.37	8.09	7.31 b†	7.80 b	7.73 c	8.58 b	0.94	0.90 b	1.96 a	1.76
Canola	9.47	8.11	7.55 a	8.03 a	8.11 a	8.74 a	0.94	0.93 a	1.92 b	1.79
Flax	9.41	8.07	7.51 a	8.02 a	7.94 b	8.69 ab	0.94	0.91 b	1.96 a	1.79
Pea	9.40	7.97	7.54 a	8.00 a	7.92 b	8.74 a	0.94	0.91 b	1.96 a	1.78
Wheat	9.39	8.02	7.46 a	8.03 a	7.95 b	8.74 a	0.93	0.91 b	1.95 a	1.78
Time										
0	9.14 b	7.85 c	6.72 c	7.63 c	7.50 c	8.93 a	0.88 c	0.8 b	1.91 c	1.61 c
48	9.52 a	8.23 a	7.40 b	8.55 a	7.93 b	8.46 b	0.87 c	0.94 a	2.01 b	1.89 a
97	9.56 a	8.06 b	8.42 a	7.59 c	7.90 b	8.48 b	1.11 a	0.93 a	2.03 a	1.89 a
362	9.42 a	8.07 b	7.34 b	8.13 b	8.39 a	8.93 a	0.90 b	0.94 a	1.84 d	1.73 b
Crop residues	NS¶	NS	**‡	**	**	*	NS	*	*	NS
Incubation Time	***	***	***	***	***	***	***	***	***	***
Crop residues x Incubation Time	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

† Different letters within the same column indicate a significant difference between means.

‡ *, **, *** Significant at $p \leq 0.05$, 0.01, and 0.001, respectively.

¶ Not significant.

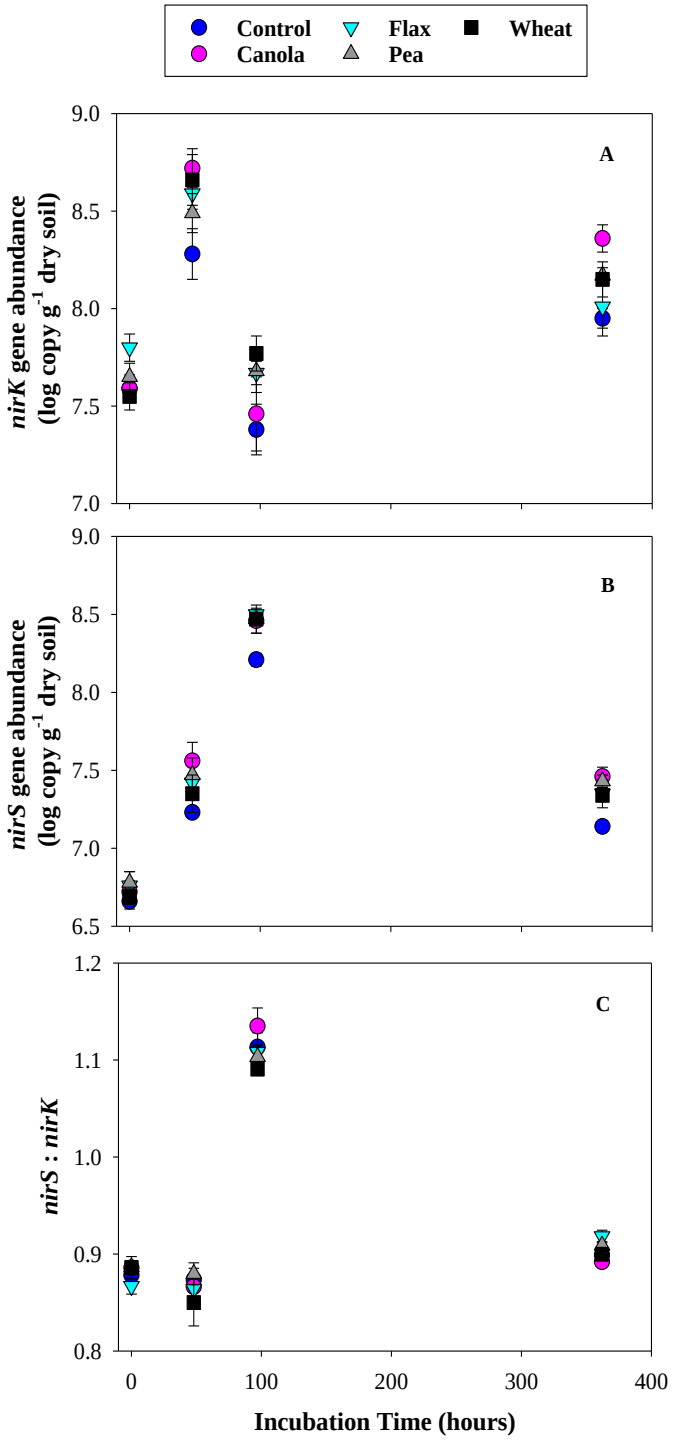

Fig 4.7 Effect of canola, flax, pea and wheat residue addition on (A) *nirK* (B) *nirS* and (C) *nirS* : *nirK* gene copy abundance. Error bars indicate standard error of the mean (n=4).

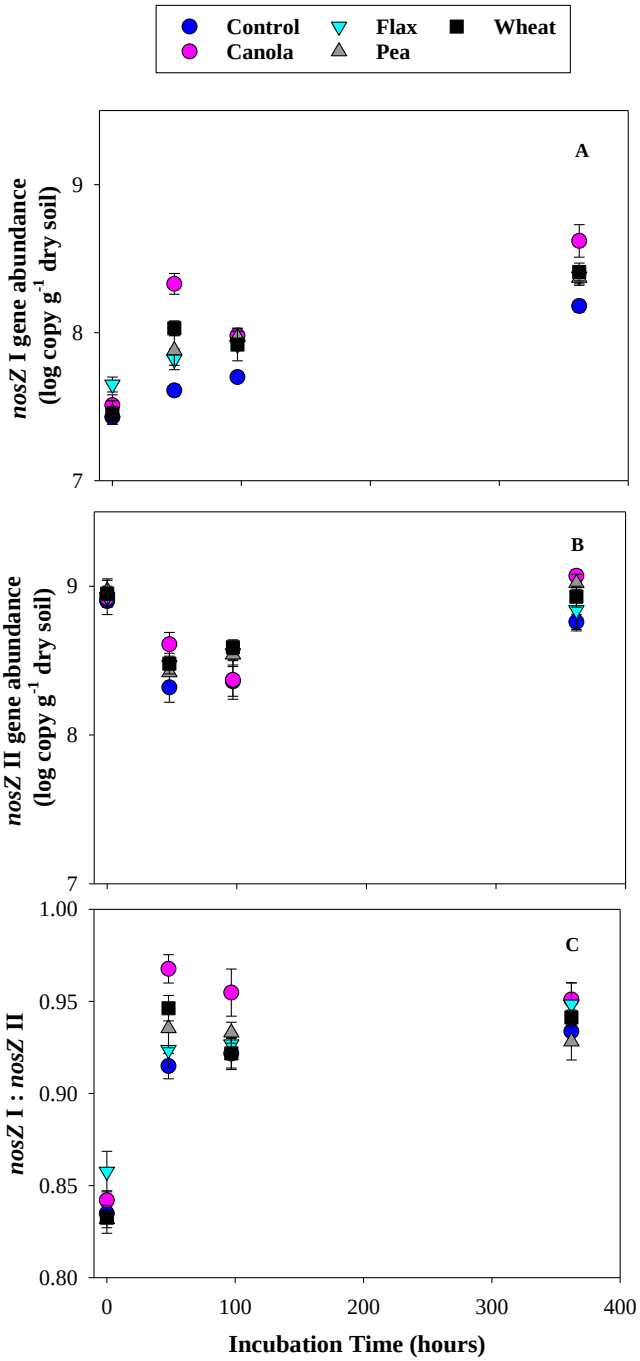

Fig 4.8 Effect of canola, flax, pea and wheat residue addition on (A) *nosZ* I (B) *nosZ* II and (C) *nosZ* I:*nosZ* II gene copy abundance. Error bars indicate standard error of the mean (n=4).

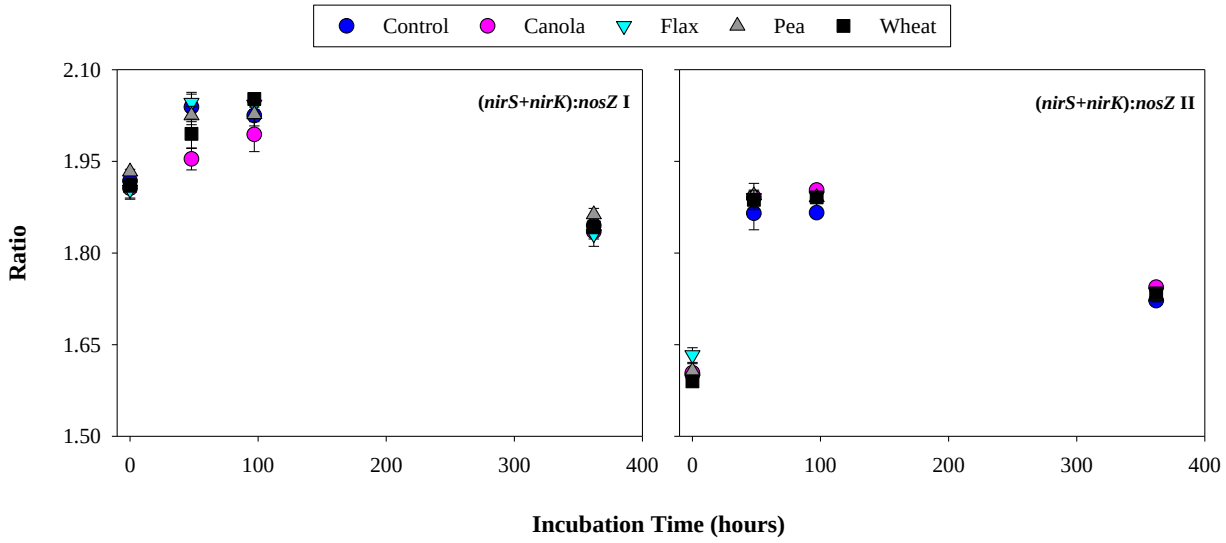

Fig 4.9 Effect of canola, flax, pea and wheat residue addition on the ratios of (A) (*nirS* + *nirK*):*nosZ* I and (B) (*nirS* + *nirK*):*nosZ* II. Error bars indicate standard error of the mean (n=4).

***Soil microbial biomass*.** Residue addition increased the abundance of total ($p < 0.001$), bacterial ($p < 0.001$) and fungal ($p < 0.001$) biomarkers throughout the incubation period compared to control and the abundance varied between residue types (Fig. 4.10, Table 4.8). Total PLFA and bacteria were consistently highest in soils amended with canola and pea residue up to 48 h after residue addition. Actinobacteria was not significantly different between residue types, but a significant increase in actinobacteria PLFA was observed on residue amended soil at 97 and 362 h compared to control (appendix, Table B.2). Also, G+ biomarker PLFA increase was observed on soils amended with canola and pea residues at 362 h compared to flax and wheat residue soils (Fig. 4.11; Table B.3). The effect of residue addition on total PLFA, bacterial, G+ and G- PLFAs all followed similar patterns and were consistently highest on soils amended with canola and pea residues, while fungal biomarkers were significantly higher on residue amended soils and varied with residue types in the order control < wheat < flax < pea < canola ($p < 0.05$) (Table 4.8). Although actinobacteria was not significantly different between soils amended with different residue types, they were greater compared to the control (Table 4.8).

Table 4.8 Repeated measures analysis of variance (ANOVA) and effects of labelled residues of canola, flax, pea and wheat application on microbial biomass in incubated soil at four different sampling times (n=4).

Crop residues	**Total PLFA**	**Bacteria**	**G +**	**G -**	**Actino.**	**Fungi**	**AMF**	**Stress 1**	**Stress 2**
Control	54.48 d†	32.81 d	13.42d	13.59 d	5.81 b	0.68 e	2.33 c	0.40 a	0.37 a
Canola	88.04 a	52.69 a	19.89a	25.85 a	6.95 a	3.19 a	2.98 a	0.32 d	0.23 d
Flax	75.32 c	44.19 c	17.68c	19.91 c	6.54 a	2.40 c	2.67 bc	0.37 b	0.28 b
Pea	84.02 ab	49.15 ab	19.12ab	23.24 ab	6.79 a	2.90 b	2.87 a	0.34 c	0.24 c
Wheat	76.67 bc	44.98 bc	17.44bc	20.99 bc	6.55 a	1.90 d	2.85 ab	0.35 c	0.26 bc
Time									
0	56.99 c	32.40 c	12.16 d	15.32 d	4.92 c	2.01 b	2.10 c	0.34 b	0.26 b
48	87.59 a	52.82 a	21.15 a	24.50 a	7.17 a	2.66 a	2.73 b	0.32 c	0.25 b
97	73.07 b	42.75 b	16.74 c	19.17 b	6.85 b	1.70 b	3.09 a	0.42 a	0.34 a
362	85.18 a	51.09 a	20.00 b	23.88 a	7.17 a	2.48 a	3.05 a	0.34 b	0.26 b
Crop residues	***‡	***	***	***	**	***	**	***	***
Incubation Time	***	***	***	***	***	***	***	***	***
Crop residues x Incubation Time	***	***	***	***	NS	NS	***	***	***

† Different letters within the same column indicate a significant difference between means.

‡ ***,**,***** Significant at $p \leq 0.05$, 0.01, and 0.001, respectively.

¶ Not significant.

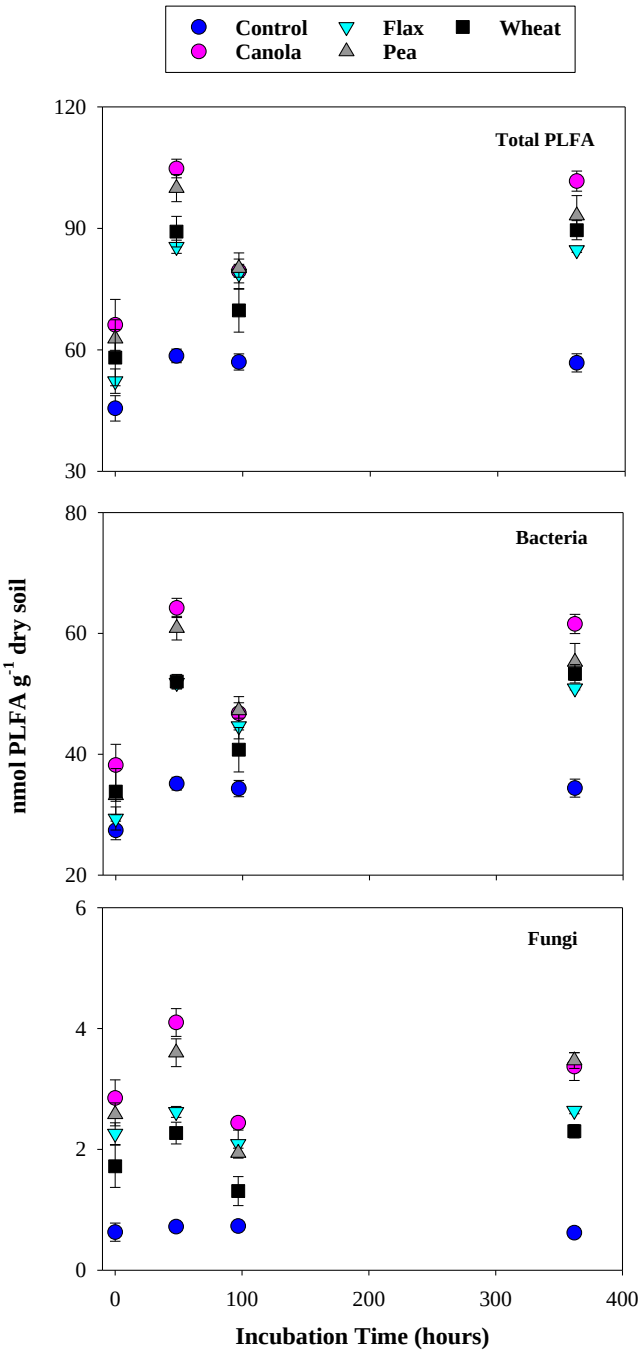

Fig 4.10 Effects of canola, flax, pea and wheat residue additions on total phospholipid fatty acid (PLFA) abundance, and bacterial and fungal biomarkers. Error bars indicate standard error of the mean (n=4).

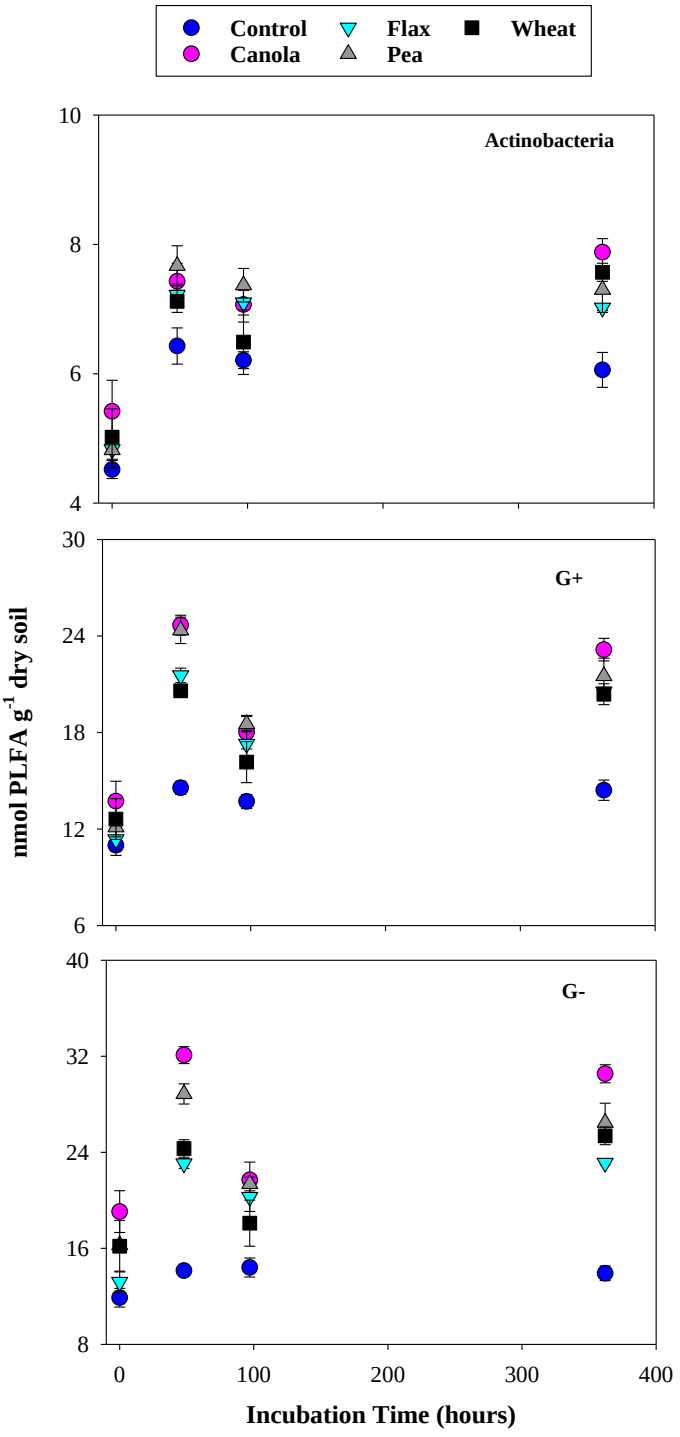

Fig 4.11 Effects of canola, flax, pea and wheat residue additions on soil actinobacteria, Gram positive (G+) and Gram negative (G-) biomarker abundance. Error bars indicate standard error of the mean (n=4).

Microbial community composition differed between residue-amended soils and the control. Microbial communities in residue-amended soils were more responsive to incubation time than different residue types (Fig. 4.12). Microbial communities in control soils were similar at all sampling point with a little shift at 97 h. Soil O_2 concentration decreased significantly between residues over the incubation period in the order canola < pea < wheat < flax < control. The decrease in soil O_2 level was moderate on control soil compared to the magnitude on residue types (see appendix, Fig. B 3).

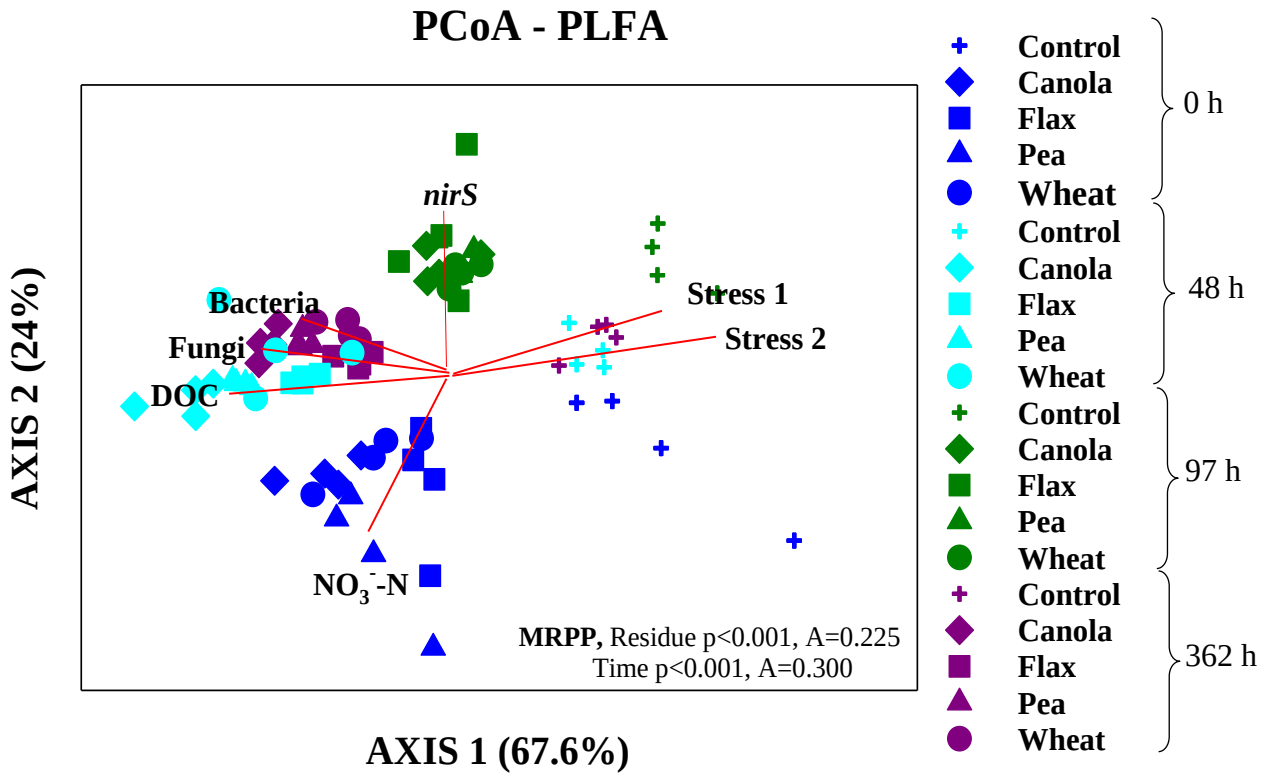

Fig 4.12 Principal coordinate analysis (PCoA) of total (^{12}C + ^{13}C) soil microbial communities in residue amended and control soils at 0, 48, 97 and 362 hours after residue addition.

Distribution of residue carbon within active microbial decomposer communities. The incorporation of ^{13}C into the bacterial, G+, G- actinobacterial and fungal PLFA biomarkers, varied significantly over time, was affected by residue type and significantly interacted with sampling time for most of the functional groups (Table 4.9). However, ^{13}C incorporation into G- bacteria and actinobacteria exhibited no residue decomposition × incubation time interaction.

Table 4.9 Repeated measure analysis of variance (ANOVA) of labelled residues of canola, flax, pea and wheat application effect on ^{13}C distribution within microbial biomass in incubated soil (n=16).

Crop residues	Bacteria	G+	G-	G+:G-	Actino	F:B	Fungi	Stress 1	Stress 2
Control	---	---	---	---	---	---	---	---	---
Canola	55.92 b†	18.23 b	32.91 a	0.61 b	3.02 b	0.40 a	17.46 ab	0.13 c	0.09
Flax	52.94 c	18.54 b	28.91 c	0.68 ab	3.32 a	0.49 a	17.98 a	0.17 b	0.11
Pea	55.68 b	20.63 a	29.88 b	0.73 a	3.31 a	0.40 ab	15.61 bc	0.20 a	0.09
Wheat	58.45 a	20.38 a	32.33 a	0.70 ab	3.81 a	0.23 b	10.75 c	0.14 bc	0.15
Incubation Time									
0	37.58 d	15.04 c	20.96 d	0.72 ab	1.58 c	1.18 a	41.16 a	0.01 b	0.05 b
48	66.76 a	15.07 c	39.88 a	0.38 c	4.07 a	0.12 b	7.69 b	0.33 a	0.04 b
97	53.63 c	25.29 a	24.75 c	1.03 a	3.58 b	0.12 b	6.23 b	0.00 c	0.28 a
362	65.03 b	22.37 b	38.45 b	0.59 b	4.22 b	0.10 b	6.74 b	0.30 a	0.07 b
Crop residues	**‡	***	***	**	**	**	**	***	NS
Incubation Time	***	**	***	**	***	***	***	***	***
Crop residues x Incubation Time	**	***	NS¶	**	NS	**	**	***	NS

† Different letters within the same column indicate a significant difference between means.
‡ ***, **, ***** Significant at $p \leq 0.05$, 0.01, and 0.001, respectively.
¶ Not significant**.**

The fungal biomarker 18:2ω6,9c assimilated the greatest proportion of residue derived ^{13}C at the earliest phase of incubation, followed by a rapid decline (Fig 4.13). The proportion of fungal PLFA biomarker that was comprised of ^{13}C differed in soils that received flax (48.6%), canola (46.2%), pea (43.6%) and wheat (26.2%) residue addition (Fig 4.13). The distribution of ^{13}C at the early stage of decomposition, specifically within actinobacteria and G+ bacteria (Fig. 4.14) was highest in soils amended with pea and wheat residues. While soils amended with wheat and canola residues are similar in G- bacterial ^{13}C distribution (Fig. 4.14), the trajectory of ^{13}C uptake shifted between 48 h and 97 h after residue application to increased ^{13}C distribution in actinobacteria and G+ bacterial biomarkers in all treatments. However, increased ^{13}C distribution was observed at 48 h into the study on G- bacteria biomarkers and the highest increase was observed on soil amended with canola residue (Fig. 4.14). The shift in ^{13}C distribution within fungal biomarker was only significant at time 0 vs 48 h ($p < 0.01$) (Table 4.9), and ^{13}C assimilation on soil amended with flax residue was significantly higher ($p = 0.02$) than all other treatments, except canola residue ($p = 0.61$) (Table 4.9). There was no difference between canola and pea residues.

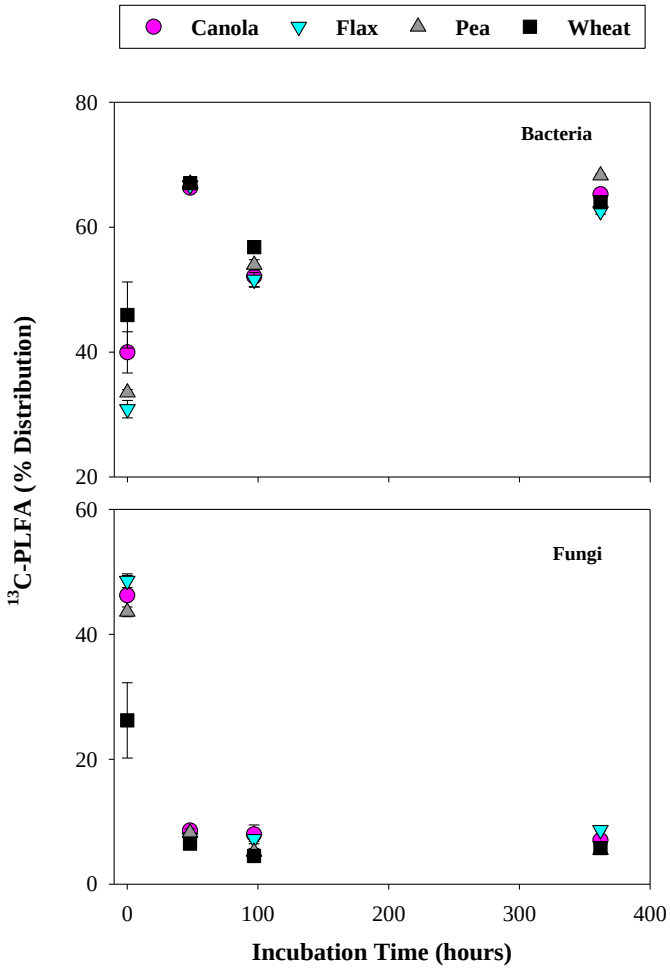

Fig 4.13 Distribution of residue ^{13}C within the phospholipid fatty acid (PLFA) biomarkers of bacteria and fungi in residue-amended soils. Error bars indicate standard error of the mean (n=4).

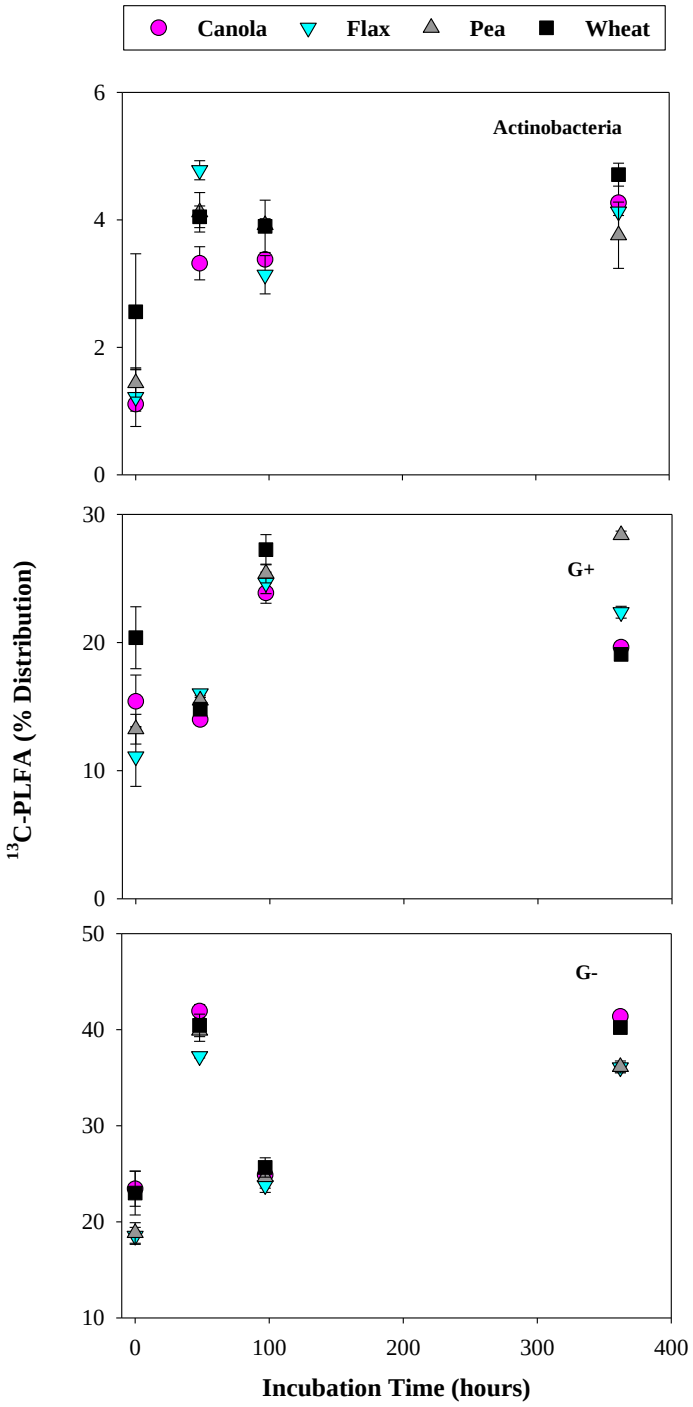

Fig 4.14 Distribution of residue ^{13}C within the phospholipid fatty acid (PLFA) biomarkers of active actinobacteria, G+ (Gram-positive bacteria), G- (Gram-negative bacteria) decomposers in residue-amended soils. Error bars indicate standard error of the mean (n=4).

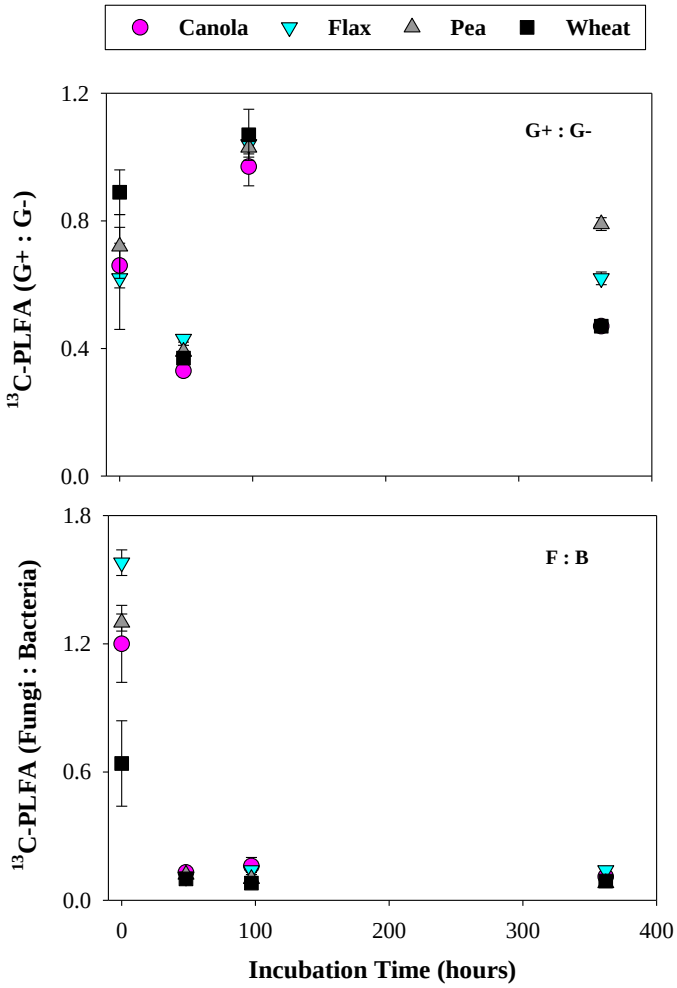

Fig 4.15 Ratio of ^{13}C distribution within the phospholipid fatty acids (PLFA) biomarkers of bacteria, fungi, G+ (Gram-positive bacteria) and G- (Gram-negative bacteria) decomposers in residue-amended soils. Error bars indicate standard error of the mean (n=4).

Similar to the overall microbial community structure, ordination of ^{13}C distribution in PLFAs showed a substantial change in the microbial community structure over time, with lesser impacts due to residue type (Fig. 4.16). The microbial communities are similar at 48 and 362 hours but are clearly distinct at 97 h which coincides with the greatest N_2O flux (Appendix B, Fig. B 1A, insert). The effect of residue application on community structure was less influential than incubation time (MRPP, residue, $p = 0.395$, incubation time $p < 0.001$) (Fig. 4.16).

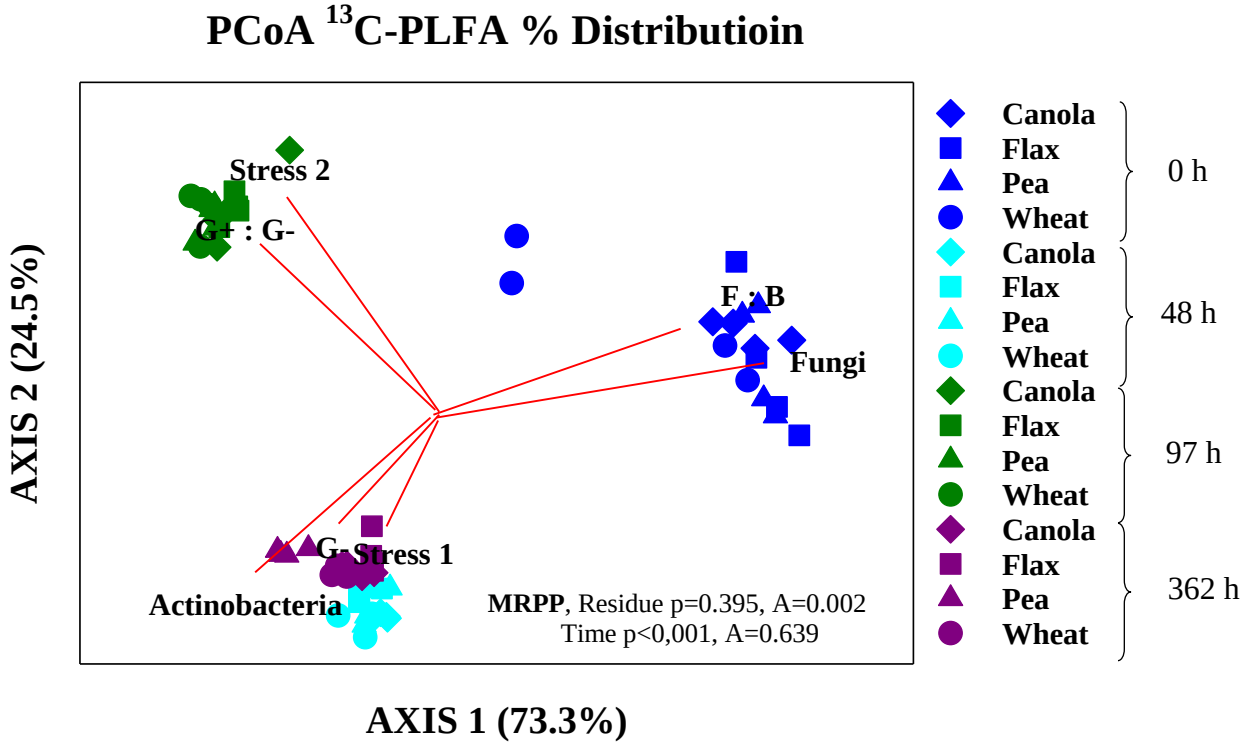

Fig 4.16 Principal coordinate analysis (PCoA) of ^{13}C distribution in soil microbial communities in residue amended soils at 0, 48, 97 and 362 h after residue addition.

When microbial community structure was analyzed separately at each sampling time, the effect of residue addition on microbial community structure became apparent (Fig. 4.17). Immediately after residue addition the microbial communities begins to separate out in the total PLFA (i.e. $^{12}C + ^{13}C$ PLFA) and based on residue C distribution within organisms assimilating residue C (^{13}C PLFA). The community structures of those organisms assimilating ^{13}C from the different residues were different to varying degrees throughout the incubation (Fig. 4.17). At 48 h, just prior to the largest measured N_2O flux, residue type resulted in clearly distinct active community structure. By 97 h when the highest N_2O flux was measured, there was less distinction (Fig. 4.17). By 362 h,

there was no distinction in total PLFA community structure. However, ^{13}C distribution within microbial communities on canola and wheat residue amended soils were similar to one another while flax and pea communities were distinct.

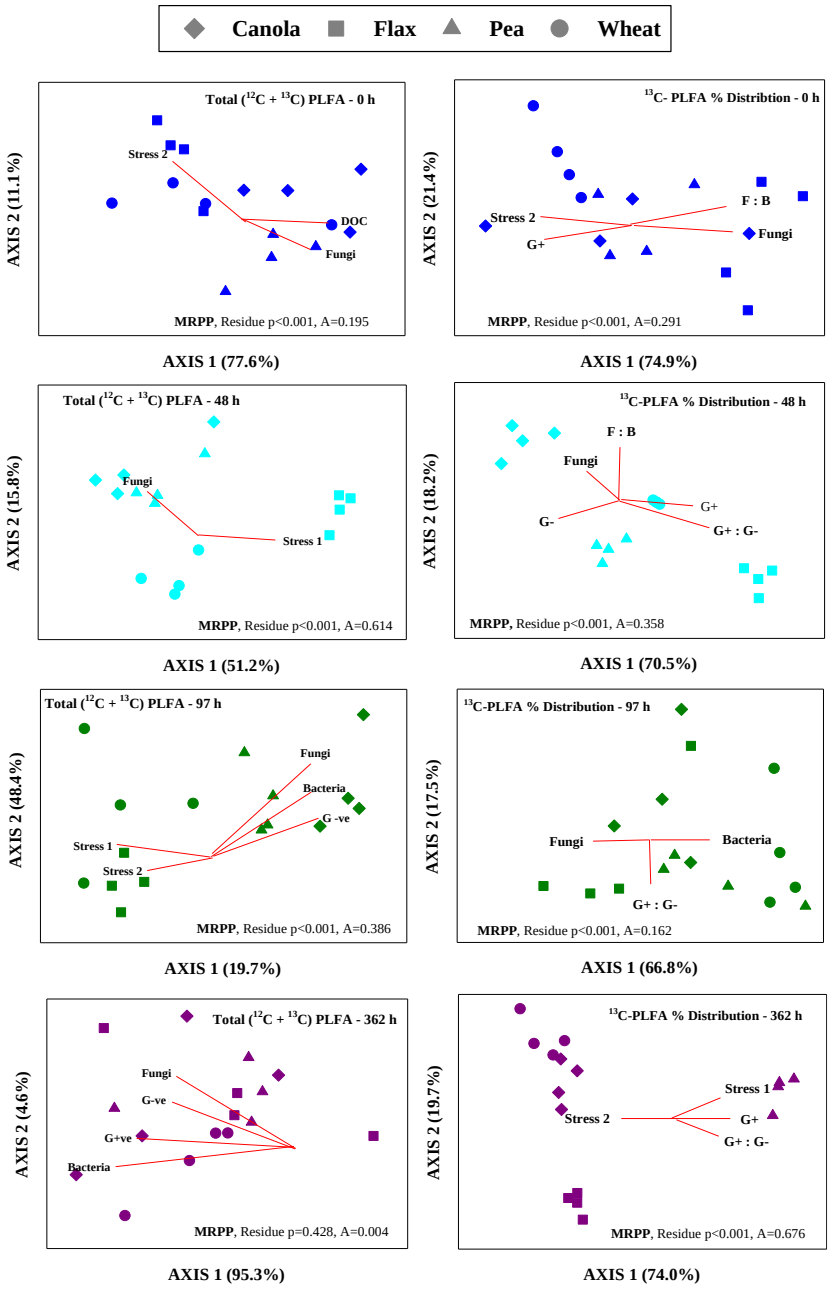

Fig. 4.17 Community structure of total (^{12}C + ^{13}C) and ^{13}C phospholipid fatty acid (PLFA) distribution in microbial biomass at 0, 48, 97 and 362 h of soil amendment with labelled residues of canola, flax, pea and wheat.

Relationships between residue addition on soil nutrient content, microbial abundance, N-cycling gene abundance and N_2O, $^{15}N_2O$,CO_2 and $^{13}CO_2$ emissions. Correlations between soil nutrients, N-cycling genes and emissions showed similar trends (Tables 4.10 and 4.11). For example, N_2O emissions and microbial respiration were negatively correlated with soluble C (DOC) (Table 4.10), and DON was negatively correlated with N_2O emissions on canola, pea and wheat but positively on control and flax (Table 4.10). Residue quality, as indicated by DOC:DON was negatively correlated with N_2O emissions on control, flax and pea but positively on canola and wheat (Table 4.10). Dissolved organic nitrogen was positively correlated with total microbial abundance and active microbial groups except on canola with actinobacteria (Appendix B, Table B3-B8).

Microbial abundance showed significant positive correlation with soil NH_4^+ availability in the control ($p < 0.05$), but was negatively correlated for flax ($p < 0.01$) (Table B3). Available NH_4^+ showed a trend of positive correlation with microbial respiration and N_2O emissions across all residue types, but was significant only for pea and wheat residue. Nitrate availability was negatively correlated with bacterial abundance across all treatments but significantly on residue-amended soils (Table B5), and also negatively correlated with N_2O, $^{15}N_2O$, CO_2 and $^{13}CO_2$ (Tables 4.10 and 4.11), whereas there was no significant correlation with fungi and NO_3^- availability (Table B4). A positive correlation between DON and fungi was significant only for canola and wheat, while a positive correlation between DON and bacteria was significant only for the control and flax (Tables B4 and B5). As well, a negative correlation between DON and N_2O and $^{15}N_2O$ emissions was significant only for canola and flax. AOA *amoA* abundance was positively correlated with N_2O emissions and microbial respiration on all treatments ($p < 0.05$), except on soil amended with canola residue (Tables 4.10 and 4.11). Denitrification gene *nirS* was positively correlated with N_2O emissions and microbial respiration in all soils. *nosZ* clade II correlated negatively with N_2O and $^{15}N_2O$ emissions on all residue-amended soils, but was significant only for the soils amended with canola and flax residues (Tables 4.10 and 4.11). On unamended soil, *nosZ* clade I positively correlated with N_2O emissions ($p < 0.01$). The ratio of denitrification genes (*nirS* and *nirk*)to *nosZ* clade I was positively correlated with N_2O emissions on all residues but not in the control. The ratio of denitrification genes (*nirS* and *nirK*) and *nosZ* clade II was positively correlated with N_2O emission across all residues and control. However, ratio of *nosZ* clade II to *nosZ*

clade I was negatively correlated with N_2O emissions on all soils (Table 4.10), suggesting that predominance of either *nos*Z clades may have substantial consequences for net soil N_2O emissions.

Table 4.10 Pearson correlations of N_2O-N and CO_2-C, with N cycling functional genes and biogeochemical properties of incubated soil, as affected by labelled residue application.

Variables	Control		Canola		Flax		Pea		Wheat	
	N_2O	CO_2	N_2O	CO_2	N_2O	CO_2	N_2O	CO_2	N_2O	CO_2
AOA *amoA*	0.553*	0.456	0.022	0.173	0.678**	0.626**	0.594*	0.732**	0.552*	0.587*
AOB *amoA*	0.264	0.217	0.114	0.257	0.436	0.263	0.348	0.483	0.121	0.115
nirS	0.501*	0.335	0.875**	0.943**	0.869**	0.935**	0.840**	0.963**	0.872**	0.923**
nirK	-0.105	-0.071	-0.453	-0.206	0.248	-0.308	-0.239	-0.170	-0.207	-0.070
nosZ I	0.884**	0.931**	-0.074	0.340	0.095	0.442	0.090	0.478	0.059	0.446
nosZ II	-0.091	0.086	-0.816**	-0.564*	-0.554*	-0.366	-0.428	-0.400	-0.321	-0.268
(*nirS*+*nirK*):*nosZ* I	-0.350	-0.513*	0.647**	0.368	0.786**	0.313	0.638**	0.368	0.652**	0.409
(*nirS*+*nirK*):*nosZ* II	0.283	0.108	0.707**	0.743**	0.930**	0.659**	0.593*	0.675**	0.556*	0.618*
nosZ II:*nosZ* I	-0.758**	-0.664**	-0.447	-0.679**	-0.483	-0.638**	-0.347	-0.657**	-0.232	-0.536*
nosZ I:nosZ II	0.762**	0.670**	0.445	0.669**	0.457	0.627**	0.339	0.650**	0.216	0.519*
DOC	-0.447	-0.475	-0.540*	-0.778**	-0.618*	-0.810**	-0.489	-0.720**	-0.369	-0.746**
DON	0.129	-0.001	-0.513*	-0.664**	0.514*	-0.015	-0.056	-0.259	-0.466	-0.598*
DOC:DON	-0.498*	-0.381	0.025	-0.071	-0.823**	-0.599*	-0.465	-0.490	0.305	0.176
GWC	-0.749**	-0.648**	-0.446	-0.773**	-0.626**	-0.687**	-0.339	-0.686**	-0.388	-0.742**
NO_3^--N	-0.814**	-0.779**	-0.430	-0.771**	-0.522*	-0.837**	-0.446	-0.767**	-0.069	-0.496
NH_4^+-N	0.420	0.261	0.239	0.112	0.151	-0.199	0.610*	0.606*	0.571*	0.461

Note: DOC & DON-Dissolved organic carbon & nitrogen, GWC- gravimetric water content, control – treatment without residue application

† *, **, Significant at $p \leq 0.05$ and 0.01, respectively.

Table 4.11 Pearson correlations of ^{15}N-N_2O and ^{13}C-CO_2, with N cycling functional genes and biogeochemical properties of incubated soil, as affected by labelled residue application.

Variables	Control		Canola		Flax		Pea		Wheat	
	$^{15}N_2O$	$^{13}CO_2$	$^{15}N_2O$	$^{13}CO_2$	$^{15}N_2O$	$^{13}CO_2$	$^{15}N_2O$	$^{13}CO_2$	$^{15}N_2O$	$^{13}CO_2$
AOA *amoA*	0.555*	0.455	-0.138	0.000	0.678**	0.506*	0.495	0.537*	0.517*	0.396
AOB *amoA*	0.263	0.233	0.009	0.144	0.426	0.254	0.261	0.297	0.133	0.018
nirS	0.506*	0.338	0.807**	0.746**	0.864**	0.818**	0.727**	0.813**	0.770**	0.760**
nirK	-0.108	-0.057	-0.601*	-0.287	0.247	-0.334	-0.266	-0.318	-0.182	-0.101
nosZ I	0.882**	0.932**	-0.162	0.360	0.083	0.514*	0.068	0.382	0.062	0.341
nosZ II	-0.094	0.077	-0.779**	-0.389	-0.544*	-0.313	-0.265	-0.359	-0.229	-0.157
(*nirS*+*nirK*):*nosZ* I	-0.346	-0.503*	0.552*	0.038	0.790**	0.152	0.524*	0.219	0.563*	0.338
(*nirS*+*nirK*):*nosZ* II	0.286	0.120	0.572*	0.496	0.922**	0.546*	0.443	0.520*	0.470	0.465
nosZ II:*nosZ* I	-0.759**	-0.672**	-0.343	-0.584*	-0.466	-0.659**	-0.228	-0.547*	-0.184	-0.385
nosZ I:nosZ II	0.762**	0.678**	0.342	0.580*	0.440	0.653**	0.219	0.547*	0.172	0.368
DOC	-0.446	-0.452	-0.469	-0.637**	-0.600*	-0.745**	-0.386	-0.623**	-0.286	-0.661**
DON	0.130	0.014	-0.609*	-0.724**	0.511*	-0.076	-0.117	-0.325	-0.424	-0.550*
DOC:DON	-0.499*	-0.384	0.176	0.101	-0.808**	-0.510*	-0.311	-0.334	0.308	0.151
GWC	-0.749**	-0.657**	-0.366	-0.626**	-0.615*	-0.652**	-0.228	-0.546*	-0.317	-0.628**
NO_3^--N	-0.815**	-0.783**	-0.349	-0.590*	-0.503*	-0.817**	-0.367	-0.653**	-0.017	-0.340
NH_4^+-N	0.421	0.273	0.072	-0.027	0.166	-0.301	0.436	0.557*	0.462	0.334

Note: DOC & DON-Dissolved organic carbon & nitrogen, GWC- gravimetric water content, control – treatment without residue application.

† *, **, Significant at $p \leq 0.05$ and 0.01, respectively.

4.6 Discussion

Results from this study show that N_2O emissions and microbial respiration depend on quantity and quality of substrate released into the soil during residue decomposition. Stable isotope probing (SIP) showed that the addition of canola residue induced substantial N_2O emissions which were sourced primarily from the soil N pool, while also inducing the short-term priming of soil organic C. Over the course of the incubation, abundance of both bacteria and fungi as well as microbial community structure responded to the addition of different residue types in a broadly similar fashion. Microbial community structure was distinct in canola residue soils at 48 h, corresponding to peak N_2O accumulation. The abundance of N functional genes was similar among residue types and did not correspond with observed differences in bacterial abundance. Residue quality-related changes in microbial abundance, microbial community structure and activity together drove N_2O emissions derived from residue and soil N pools.

Microbial carbon and nitrogen use following residue addition.

Crop residue incorporation into the soil can shift microbial abundance, community structure and activity with important consequences for biogeochemical processes, including gaseous N losses. In this study, addition of different crop residue types induced an increase in N_2O emissions originating from the soil N pool (i.e., RISE; Table 4.3) and induced positive short-term priming of SOC — with the magnitude of the priming effect varying among residue types (Table 4.5). Residue additions also produced an increase in labile C utilization, and a corresponding increase in microbial abundance that was greater for canola residue compared to the other residue types (Fig 4.10). Several studies have linked C substrate quality and availability to soil microbial biomass turnover (Nguyen and Marschner, 2016; Leifeld and von Lützow, 2014) as a major determinant of the magnitude and direction of the priming effect (Shahbaz et al., 2017; Graf et al., 2014). In the present study, canola residue decomposition was characterized by a longer intensive C mineralization phase than either pea or wheat residues (Table 4.4) and resulted in the greatest increase in bacterial and fungal biomass. An increase in available C also stimulates microbial metabolism, with a concomitant increase in O_2 consumption that can create conditions favorable for denitrification (Miller et al., 2008; Beauchamp et al., 1989). Indeed, an increase in microbial respiration—together with a corresponding O_2 depletion—was observed in the residue-amended soils and was greater in soil amended with canola residue (Appendix B, Fig B.3) which yielded the largest amounts of N_2O.

Nitrogen mineralization was significantly higher in the residue-amended soils than in the unamended control soil, and there were significant differences among residue types. Lag periods differed between $^{13}CO_2$ and $^{15}N_2O$ emissions following canola, pea and wheat residue addition (Tables 4.2 and 4.4) and increasing DOC-to-DON ratio (Fig 4.5C) indicated that soil microorganisms quickly became N limited as residue decomposition progressed with increasing microbial abundance and demand for N. Due to increased microbial respiration and O_2 depletion, the demand for alternative terminal electron acceptors increased, and NO_3^-/NO_2^- preference over N_2O has been documented (Miller et al., 2008) thus increasing accumulation and subsequent N_2O emissions. Nitrous oxide emissions following residue addition can vary due to differences in the C:N ratio or decomposability of the crop residue (Duan et al., 2018). ^{15}N isotopic tracing showed that most of the N_2O in soils with canola residues came from the soil N pool indicating that canola residue decomposers were mining N from the SOM. Increased heterotrophic bacterial NO_3^- consumption and decreased DOC indicates that the microbial community was using readily available C, and all these scenarios pointed to denitrification as the likely source of N_2O emissions.

Of the four residue types used in this study, canola, pea and wheat had C:N ratios that were much greater (ranging from 35:1 to 49:1) than that of flax, which had a C:N of only 27:1 (Table 4.1). Additions of fresh crop residue stimulate copiotrophic microorganisms that are characterized by high rates of growth and respiration (Pascault et al., 2013; Bastian et al., 2009). In contrast, oligotrophic microbial populations are characterized by lower rates of growth and respiration, and become increasingly dominant as the availability of labile C decreases during residue decomposition (Fanin et al., 2019; Fanin and Bertrand, 2016). Kramer and Gleixner (2008) used stable isotope analysis to explain a shift in the proportion of G+ and G- bacteria based on C availability. In line with this idea, many studies have shown an increase in the ratio of G+ to G- bacteria with decreasing C availability following exhaustion of labile substrate (Fanin et al., 2019; Breulmann et al., 2014). This is in agreement with our findings where the G+:G- bacteria ratio increased more on pea and wheat residues compared to canola residue. Likewise the physiological stress biomarkers were lowest for the canola residue soils. This is likely attributed to prolonged availability of C from canola compared to the other residues. In contrast, there was no lag period for N mineralization in flax residue amended soils, which had the lowest cumulative N_2O and $^{15}N_2O$ emissions compared to other residue treatments, despite having a narrow C:N ratio (Table 4.1). Delayed residue C availability from flax (Table 4.4) and a consequent delay in microbial conversion of

already available mineral N and/or immobilization of N from flax residue may explain lower N_2O emission from soil with flax residue compared to other residues.

Large N_2O emissions in canola residue amended soils were associated with the highest level of CO_2 respiration and concomitant decreases in DOC and DON. This increased activity and utilization of DOC and DON corresponded with higher microbial abundance, as well as higher residue-derived ^{13}C incorporation into bacteria and fungi compared with other residue types. All residue-amended soils produced larger amounts of N_2O compared to the unamended control soil, which agrees with previous studies that show increased N_2O emission after crop residue addition (Gao et al., 2016; Baggs et al., 2006; Huang et al., 2004). Microbial activity following the incorporation of crop residues is influenced by the quality of native SOM and of the residue inputs themselves (Moreno-Cornejo et al., 2015) as well as to changes in microbial community structure that affects soil processes (Cavigelli and Robertson, 2001). Differences in N_2O emissions between residue types were likely due to residue quality characteristics. For example the soil inorganic N (NH_4^+ and NO_3^-), DOC and DON contents were different between residue types (Figs 4.4 and 4.5).

N functional gene response to residue addition.

In agreement with previous studies (Gao et al., 2016; Tatti et al., 2014), I observed that the addition of crop residues significantly increased *nirS* and *nirK* gene copy numbers compared to the control. However, there was no differences in *nirS* and *nirK* genes copies between the different crop residue treatments themselves. Residue addition also increased *nos*Z gene clade I and II ($p < 0.05$), and canola *nos*Z I gene abundance was significantly higher compared to other residues at 48 h ($p = 0.05$). This provides evidence that different residues did not simply increase the number of *nirS* and *nirK* containing organisms. Instead, residue quality influenced N_2O emissions through differences in activity and potentially identity of organisms producing and consuming N_2O. All steps in the denitrification pathway are not necessarily performed by same organism (Graf et al., 2014), but the genetic potential for N_2O production and consumption within a denitrifying community can inform the balance of N_2O production and consumption. As in Tosi et al. (2020) and Wu et al. (2017), I observed a positive relationship between *nirS* and N_2O emissions. However, I also observed a negative relationship between cumulative N_2O and *nirK*, similar to what has been reported by Jones et al. (2014) and Németh et al. (2014). The *nirK* and *nirS* denitrifiers responded

to change in substrate availability (DOC and DON) as the residues decomposed, which is consistent with differences in residue type/quality. However, the *nirK* seem to be more sensitive to these nutrient changes than *nirS* denitrifiers, in agreement with Bárta et al. (2010) and Kandeler et al. (2006). Contrasting response between *nosZ* clades was observed, and niche differentiation of the two clades in response to environmental conditions remains obscured (Hallin et al., 2018). Our study shows that *nosZ* I:*nosZ* II increased with O_2 limitation and increased consumption of DOC, DON and NO_3^- that was most evident in soil amended with canola residue.

Fungal and bacterial contributions to N_2O emissions.

A high incorporation of residue C into the fungal biomass, was reported in previous studies (Arcand et al., 2016; Helgason et al., 2014) and highlights the importance of fungi in the early stages of crop residue decomposition. The percentage of residue C distributed in the fungal biomarker was the same among residue types at all times except time 0 (Fig. 4.13) while total fungal PLFA was consistently higher in soil amended with canola residue (Fig. 4.10)—indicating higher assimilation of canola residue C into fungal biomass. Fungal denitrification may contribute more to soil N_2O emission than bacterial denitrification (Phillips et al., 2016; Chen et al., 2015) because fungi lack N_2O reductase gene (*nosZ*) (Chen et al., 2014b; Zhou et al., 2001; Zumft, 1997). Unlike denitrifying bacteria that require strict anaerobic conditions for NO_3^- or NO_2^- reduction, fungi can perform denitrifying function under anaerobic or microaerobic conditions (Takaya, 2009; Zhou et al., 2001; Zumft, 1997).

The increase in microbial abundance associated with residue additions (Table 4.8, Fig 4.10) followed a similar pattern with denitrification gene copy abundance (Table 4.9, Figs 4.7 & 4.8) though the magnitude of increases were not the same. This shows that an increase in microbial populations as result of residue addition does not result in corresponding increases in N cycling genes which was corroborated by differences in community structure. There was a significant increase in denitrification gene abundance during the incubation (Table 4.8) reflecting the impact of soil O_2 status which is important for microbial utilization of NO_3^-/NO_2^- as terminal electron acceptor. Fungal denitrification of NO_3^- and/or NO_2^- in response to the oxidation of organic C has been confirmed (Tsuruta et al., 1998; Shoun et al., 1992; Shoun and Tanimoto, 1991). Bacterial and fungal co-denitrification has been reported at O_2 concentrations that are low (*ca*. 2% by volume)

and cannot support aerobic respiration, and when two N sources (inorganic and organic) are combined (Long et al., 2013; Spott et al., 2011; Su et al., 2004). These are likely scenarios in this study with lower DOC and increasing CO_2 emission corresponding with decreasing O_2, and decreasing DON and NO_3^- observed in soils amended with residues.

4.7 Conclusion

Adding $^{15}N/^{13}C$ labelled residue to the soil enabled us to follow the fate of residue C and N to identify drivers of N_2O emissions in residue-amended soil. The size of the microbial biomass, the magnitude of microbial activity and N_2O emissions derived from the soil N pool depends on residue quality (i.e. labile C availability). This study shows that reside C quality influenced the abundance and the community structure of residue decomposers with implications for N_2O emission. Residue contribution to N_2O emissions depends on labile C availability after residue addition, which is determined by residue decomposability and nutrient release into the soil medium.

Findings from this study demonstrated that microbial preference for canola residue as a result of higher C availability stimulated growth and activity which consequently led to N deficiency. The use of stable isotope showed that most N_2O-N came from soil N pool, and ^{13}C-PLFA revealed that microbial community structure in canola residue amended soil was distinct during peak N_2O emissions. Also, N cycling genes were not different between residue types, showing that higher N_2O emission from canola is not directly linked to increased abundance of *nirS* and *nirK* containing organism, but fungi or condition (low O_2) due to high aerobic respiration in soils amended with canola residue. Other studies involving canola root exudates, seed meal and green manure effect on soil microorganism showed contrasting result to this study, thus our study indicates that the dynamics of dry canola stems, leaves and roots effects on microbial activity and processes in the soil is different and promote N_2O emission through enhanced denitrification. Thus the understanding of microbial preference for canola residue particularly fungi and effects on N cycling in agricultural soils, will assist in planning N fertilization and N use efficiency in crops following canola in rotation and prevention of environmental degradation as a result of N loss as N_2O emissions.

5. CONCLUSION AND FUTURE RESEARCH NEEDS

Climate change is an important environmental issue and soil-derived N_2O emissions have the potential to exacerbate the situation. Nitrous oxide production in soils involves the microbially mediated processes of nitrification and denitrification. Denitrification often includes reduction of N_2O to N_2 which completes the N-cycle (Hallin et al., 2018). Denitrifiers either lacking genetic capacity for N_2O reduction (*nosZ*) or environmental factors affects complete denitrification, resulting in formation of N_2O as the terminal product (Graf et al., 2014; Philippot et al., 2011). Indeed, the large quantities of N added to agricultural soils as fertilizer (both inorganic and organic) are major contributors of atmospheric N_2O via soil-based N transformations (Butterbach-Bahl et al., 2013). In the quest to feed an ever increasing population, agriculture has relied heavily on use of N fertilizers to increase crop yields, which in turn has resulted in a concomitant increase in the volume of crop residues left in the field to decompose following crop harvest. These crop residues serve as readily available organic C and N sources which are critical for maintaining soil organic matter and fertility but have the potential to stimulate greenhouse gas emissions, including N_2O. Agricultural activities including fertilizer use and crop residue decomposition contribute about 77% of Canadian anthropogenic N_2O emissions (Environment and Climate Change Canada, 2018).

The magnitude of soil-derived N_2O emissions is affected by a variety of factors including soil type, climate, agricultural management practices (irrigation, residue retention, crop rotation, tillage) and environmental conditions (Lemke et al., 2018, 1998; Rochette, 2008a; Gregorich et al., 2005; Helgason et al., 2005). Consequently, developing sustainable production practices to meet increasing demands for food and fiber and reduce the impact of GHG emissions are challenges facing today's farmers. Understanding the microbial processes of N transformation in the soil will lead to the development of best management practices aimed at mitigating this problem. In Canada, over 8.9 million ha of canola was harvested in 2018 of which Saskatchewan produced more than 50% (Statistics Canada, 2019). With the potential for increase in canola production and residue generation through irrigation and increased N fertilizer use in the semi-arid climatic zone, there is the need to understand the resultant N_2O emissions. Recent studies had shown that N_2O emissions are generally higher during crop years following canola, so the question is why, or what is it about canola that contributes to higher N_2O emissions.

5.1 Summary of Findings

The first study in this thesis (Chapter 3) focused on the effect of N fertilizer application on N_2O emissions in irrigated canola. The objectives of this study were to (*i*) determine if the rate and timing of the N application affects the activity of the enzymes involved in N transformations in the soil, N cycling genes, and/or N_2O emissions, and (*ii*) relate N application rate and timing to the abundance of N-cycling genes during a two-year field study. Two N application rates (110 and 220 kg N ha^{-1}) were applied as urea (46-0-0), either 100% broadcast and incorporated at seedling or as a split application with 50% applied at seeding (55 and 110 kg N ha^{-1}) and the remaining 50% applied pre-bolting as top dress. The field experiment—which was carried out at CSIDC in Outlook, SK to examine how 4R nutrient management affected N_2O emissions in irrigated canola—provided a unique opportunity to examine microbial factors involved in nitrification and denitrification both before and after the application of N and water (i.e., irrigation), and assess relationships between these microbial factors and N_2O emissions associated with the sampling events.

The pattern and intensity of N_2O emissions differed between years, with larger emissions occurring in 2015 which was drier with fewer rewetting cycles (Fig 3.2) compared to 2016 (Fig 3.3). This impacted microbial diversity, evenness and richness, and consequently N-cycling processes (Banerjee et al., 2016). In 2016, with wetter soil conditions, N conversion enzyme (urease and arylamidase) activity was higher compared to 2015—which hastened urea hydrolysis and subsequent nitrification as shown by an increase in soil N (NH_4^+ and NO_3^-) availability. The NO_3^-N was available for plant uptake or microbial turnover, and corresponded with increased microbial activity measured as an increase in arylamidase activity. A decrease in the abundance of AOA *amoA* (nitrifiers) following irrigation in 2016, indicted that there was reduction in the potential for nitrification which would limit the primary substrate for N_2O production in soils (i.e., NO_3^-). In 2015 however, potential nitrification increased after irrigation, with a corresponding increase in nitrifier abundance. In both years, the largest N_2O emission occurred after irrigation, showing that N_2O emission in 2015 was driven by nitrifier-denitrification, due to an observed increase in abundance of AOB *amoA* and *nirS* gene copies. In 2016, N_2O emissions after irrigation were mainly driven by denitrification as indicated by increased *nirK* gene abundance, higher soil water content and reduced O_2, favoring microbial use of NO_3^-/NO_2^- for respiration under anaerobic conditions. The highest N_2O emissions were observed when 220 kg N was applied either all at seeding (BC-220) or when half was applied at seeding and the remaining half at bolting. Conversely timing of N

application did not influence N_2O emission, instead N_2O emission is influenced by increasing rate of N application. Interestingly, urease activity decreased at higher N application rates, indicating that urea conversion might be delayed and affect N availability for immediate plant uptake but later contribute to N_2O emission when conditions (such as moisture availability) become favorable. Thus, soil conditions before N application and irrigation significantly affect N_2O production pathways and magnitude of N_2O emission, and should be taken into consideration when planning irrigation application. High rate of N application, if not beneficial to yield increase, should be avoided.

The second study in this thesis (Chapter 4) focused on investigating why canola residues induce higher than expected N_2O emission. I used $^{15}N/^{13}C$ labelled residues of canola, flax, pea and wheat to quantify residue-derived and residue-induced N_2O and CO_2 emissions. The abundance of microbial N-cycling genes, microbial abundance and microbial community structure were quantified to link N_2O emissions with microbial abundance and activity to better understand the causal links between residue decomposition dynamics and N_2O emissions.

Results from this study showed that compared to the unamended soils, residue addition stimulated N_2O and CO_2 emissions from soils, which was in agreement with previous studies (Begum et al., 2014; Chen et al., 2013). Crops residues provide a source of readily available C and N in the soil and stimulate microbial activity. Among the residue types, canola residue had the highest emission factor (14.2%), and yielded more N_2O and CO_2 directly from the residue (i.e., RDE but most of the N_2O-N was derived from soil N pools (i.e., RISE). Easily decomposable C in canola residues stimulated both bacterial and fungal growth and activity. Residue C use quickly resulted in N limitation which drove both C and N priming from SOM. Concomitant consumption of O_2 led to the formation of anaerobic microsites, thus favoring denitrification and N_2O emission (Kesenheimer et al., 2019; Li et al., 2013b). ^{13}C-SIP showed the formation of a distinct active microbial community in canola residue amended soils that corresponded with peak N_2O emissions. The distribution of ^{13}C in the PLFAs of microbial biomass showed that canola residue likely decomposed faster than other residues, thus supplying more DON that consequently underwent nitrification and/or denitrification. Both bacterial and fungal growth were stimulated by residue addition, with greatest fungal ^{13}C assimilation from canola residue. Again, the higher decomposition

rate of the canola residue produced more DOC, serving as a substrate for increased microbial activities, leading to O_2 depletion and favoring an increased denitrification and a shift in microbial community structure. Higher fungal abundance and canola residue C assimilation indicates the potential for fungal denitrification which may have contributed to higher N_2O emissions from canola residue-amended soil (Chen et al., 2015). As such, understanding the effects of management practices (N fertilization and irrigation), and canola residue on soil microbial N dynamics, will help to mitigate N loss as N_2O emission in canola production system and improve N use efficiency.

5.2 Future Research

This is the first study to determine how N_2O emissions under irrigated canola in Saskatchewan are affected by management practices (i.e., the rate and timing of fertilizer N application) and relate this to changes in microbial activity. Despite providing evidence linking N_2O to specific management practices in canola production, this study was performed in the Dark Brown soil zone. Canola is grown across many soil zones in Saskatchewan there is still the need to establish a link between N_2O and specific canola management practices in other soil zones. Higher fungi abundance and ^{13}C distribution in the fungal biomarkers on canola residue-amended soil might be pointing to fungi contribution to N_2O emission in canola production systems, further research on fungi contribution to N-cycling in canola production or canola rotation system might yield better understanding on why N_2O emission was higher in systems involving canola residue. As pointed out in our study, other studies involving canola root exudates, seed meal and green manure effect on soil microorganism showed contrasting result, also studies had shown higher emission from crops following canola on the field, thus understanding the effect of different form (dry or green manure), and parts of canola residue left behind on the field on microbial activity and processes in the soil will fully explain higher or variance in N_2O emission from canola production systems.

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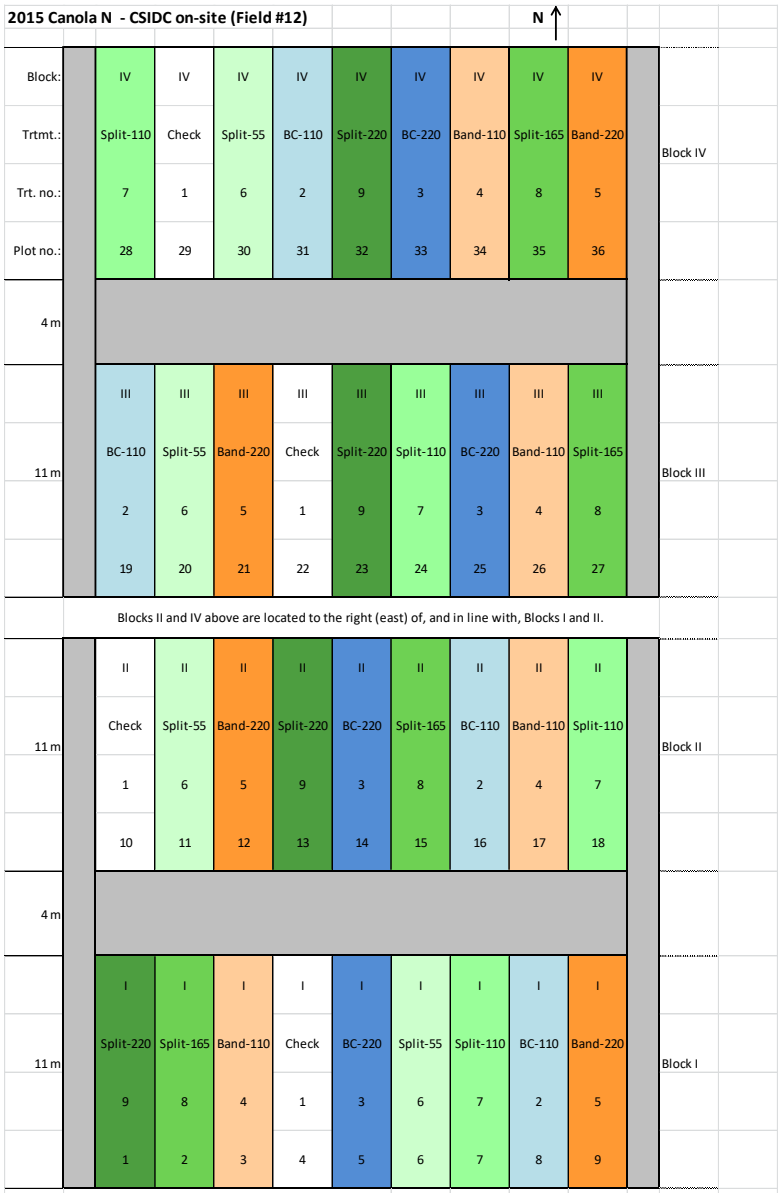
APPENDIX A

Irrigated canola field study map and correlation data



Treatment List

Trtmt. No.	Designation	Description
1	Check	No N applied
2	BC-110	110 kg/ha N pre-plant broadcast and incorporated
3	BC-220	220 kg/ha N pre-plant broadcast and incorporated
4	Band-110	110 kg/ha N sidebanded at planting
5	Band-220	220 kg/ha N sidebanded at planting
6	Split-55	27.5 kg/ha N pre-plant broadcast and incorporated plus 27.5 kg/ha N topdress just prior to bolting
7	Split-110	55 kg/ha N pre-plant broadcast and incorporated plus 55 kg/ha N topdress just prior to bolting
8	Split-165	82.5 kg/ha N pre-plant broadcast and incorporated plus 82.5 kg/ha N topdress just prior to bolting
9	Split-220	110 kg/ha N pre-plant broadcast and incorporated plus 110 kg/ha N topdress just prior to bolting

Fig. A1. Outlook field study map.

A1. Detail Description of qPCR Methods and thermocycler conditions.

Abundance of archaeal *amoA* was performed using serial plasmid dilutions carrying the AOA *amoA* gene copy numbers ranging from 1×10^3 to 1×10^9 gene copies μL^{-1} as standards. Quantitative PCRs were performed in three technical replicates for each sample. The qPCR master mix with a total volume of 20 µL contained 0.25 µL of 10 µM of each primer (crenamoA 23 and crenamoA 616), 0.4 µL of 1:10 Rox reference dye, 0.4 µL of 50 mM $MgCl_2$, 0.63 µL of 1:5 10 mg BSA, 10 µL of Platinum SYBR 2X mix (Invitrogen, Burlington, ON), 4 µL of template DNA 10 $\mu g \cdot \mu L^{-1}$ and 4.08 µL of ultra-pure H_2O. Real-time PCR cycling conditions included an initial UDG incubation step at 50 °C for 2 min and an enzyme activation step at 95 °C for 2 min followed by 45 cycles of 95 °C for 1 min, annealing at 55 °C for 1 min, and extension at 72 °C for 1 min. Acquisition of fluorescence signal was performed in an additional step of 80 °C for 1 min at the end of each cycle. Melting curve analysis consisted of 95 °C for 15 s, 60 °C for 1 min, and 95 °C for 15 s.

Quantification of bacterial *amoA* gene copy number was performed using serial plasmid dilutions carrying the AOB *amoA* gene copy numbers ranging from 10^2 to 10^8 gene copies μL^{-1} as standards. Quantitative PCRs were performed in three technical replicates for each sample. The qPCR master mix with a total volume of 25 µL contained 1.5 µL of 10 µM of each primer (amoA 1F and amoA 2R), 0.5 µL of 1:10 Rox reference dye, 0.78 µL of 1:5 10 mg BSA, 12.5 µL of Platinum SYBR 2X mix (Invitrogen, Burlington, ON), 4 µL of template DNA 10 $\mu g \cdot \mu L^{-1}$ and 4.22 µL of ultra-pure H_2O. Real-time PCR cycling conditions included an initial UDG incubation step at 50 °C for 2 min and an enzyme activation step at 95 °C for 2 min followed by 46 cycles of 95 °C for 45 s, annealing at 55 °C for 45 s, and extension at 72 °C for 45 s. Acquisition of fluorescence signal was performed in an additional step of 80 °C for 1 min at the end of each cycle. Melting curve analysis consisted of 95 °C for 15 s, 60 °C for 30 s, 0.3 °C with data collection toggled on and 95 °C for 15 s.

Abundance of *nirS* gene was quantified by using plasmid serially diluted, carrying the *nir*K genes ranging from 10^3 to 10^9 gene copies μL^{-1} as standards. Quantitative PCRs were performed in three technical replicates for each sample. The qPCR master mix with a total volume of 25 µL contained 2.50 µL of 10 µM of forward primer (cd3aF), 2.50 µL of 10 µM of reverse primer (R3cd), 0.5 µL

of 1:10 Rox reference dye, 0.78 µL of 1:5 10 mg BSA, 12.5 µL of Platinum SYBR 2X mix (Invitrogen, Burlington, ON), 5 µL of template DNA 10 $\mu g \cdot \mu L^{-1}$ and 1.22 µL of ultra-pure H_2O. Real-time PCR cycling conditions included an initial UDG incubation step at 50 °C for 2 min, and an enzyme activation step at 95 °C for 2 min followed by 6 cycles of (95 °C for 15 s, 63 °C for 30 s, and (touchdown of- 1 °C per cycle), 72 °C for 30 s, 80 °C for 15 s with data toggled on), and 40 cycles of (95 °C for 15 s, 58 °C for 30 s, 72 °C for 30 s, 80 °C for 30 s with data toggled on). Melting curve analysis consisted of 95 °C for 15 s, 60 °C for 15 s, 0.5 °C with data collection toggled on and 95 °C for 15 s.

Abundance of *nirK* gene was quantified using plasmid dilutions carrying the *nirK* gene from 10^2 to 10^8 gene copies μL^{-1} as standards. Quantitative PCRs were performed in three technical replicates for each sample. The qPCR master mix with a total volume of 25 µL contained 1.5 µL of 10 µM of forward primer (nirKH1F) and 2.0 reverse primer (nirKH1R), 0.5 µL of 1:10 Rox reference dye, 0.78 µL of 1:5 10 mg BSA, 12.5 µL of Platinum SYBR 2X mix (Invitrogen, Burlington, ON), 3 µL of template DNA 10 $\mu g \cdot \mu L^{-1}$ and 4.72 µL of ultra-pure H_2O. Real-time PCR cycling conditions included an initial UDG incubation step at 50 °C for 2 min and an enzyme activation step at 95 °C for 2 min followed by 6 cycles of (95 °C for 15 s, 65 °C for 30 s and (touchdown of- 1 °C per cycle), 72 °C for 30 s, 80 °C for 15 s with data toggled on), and 40 cycles of (95 °C for 15 s, 60 °C for 30 s, 80 °C for 15 s with data toggled on). Melting curve analysis consisted of 95 °C for 15 s, 60 °C for 15 s, 0.5 °C with data collection toggled on and 95 °C for 15 s.

The *nosZ* gene copies abundance was quantified using plasmid dilutions carrying the *nos*Z gene copies ranging from 10^2 to 10^3 gene copies μL^{-1} as standards. Quantitative PCRs were performed in three technical replicates for each sample. The qPCR master mix with a total volume of 25 µL contained 2.5 µL of 10 µM of each primer (*nosZ*2F and *nosZ*2R), 0.5 µL of 1:10 Rox reference dye, 0.78 µL of 1:5 10 mg BSA, 12.5 µL of Platinum SYBR 2X mix (Invitrogen, Burlington, ON), 3 µL of template DNA 10 $\mu g \cdot \mu L^{-1}$ and 3.22 µL of ultra-pure H_2O. Real-time PCR cycling conditions included an initial UDG incubation step at 50 °C for 2 min and an enzyme activation step at 95 °C for 2 min followed by 6 cycles of (95 °C for 15 s, 65 °C for 30 s and (touchdown of- 1 °C per cycle), 72 °C for 30 s, 80 °C for 15 s with data toggled on), and 40 cycles of (95 °C for 15 s, 60 °C for 30 s, 80 °C for 15 s with data toggled on). Melting curve analysis consisted of 95 °C for 15 s, 60 °C for 15 s, 1 °C with data collection toggled on and 95 °C for 15 s.

Table A1. Description of qPCR standards and primers

Gene	Source	Product size (bp)	Primers	References
Archaeal amoA	*Fosmid 54d9*	624	CrenamoA23F, CrenamoA616R	(Tourna et al., 2008)
Bacterial amoA	*Nitrosomona europa*	~450	amoA1F, amoA2R	(Rotthauwe et al., 1997; Stephen et al., 1999)
nirS	*Pseudomonas stutzei*	410	Cd3aF, R3cdR	(Throbäck et al., 2004)
nirK	*Sinorhizobium meliloti*	473	nirKH1F, nirK1R	(Dandie et al., 2011)
nosZ I	*Pseudomonas stutzei*	259	nosZ2F, nosZ2R	(Henry et al., 2006)
nosZ II	*Pseudomonas stutzei , Environmental samples*	267	nosZ1F, nosZ1R	(Henry et al., 2006)

Table A2. Spearman correlations of N_2O emissions with GWC and enzyme activity as affected by rate and timing of N application in soil under irrigated canola in 2015 and 2016

Year	Timing	Rate	GWC	DEA	Urease	Arylamidase	P. nitrification
2015	Control	Control	0.249	0.315	0.059	-0.488*	0.088
	Broadcast	BC-110	0.237	0.457	0.132	-0.532*	0.398
		BC-220	0.152	0.277	-0.110	-0.512*	0.598**
	Split	Split-110	0.685**	0.597*	-0.133	-0.472*	0.541**
		Split-220	0.817**	0.358	-0.121	-0.475*	0.565**
2016	Control	Control	0.381	-0.208	0.061	-0.265	0.172
	Broadcast	BC-110	0.305	0.347	0.075	0.015	0.165
		BC-220	0.261	0.337	-0.248	-0.056	0.046
	Split	Split-110	0.614**	0.722**	0.132	-0.201	0.301
		Split-220	0.700**	0.833**	0.151	-0.164	0.253

Note: GWC-Gravvimetric water content, DEA – Denitrifying enzyme activity and P – Potential.
*, **, Significant at $p \leq 0.05$ and 0.01 respectively.

Table A3. Spearman correlations of N_2O emissions with NO_3^--N and NH_4^+-N as affected by rate and timing of N application in soil under irrigated canola in 2016

Year	Timing	Rate	NO_3^--N	NH_4^+-N
2016	Control	Control	0.473*	-0.334
	Broadcast	BC-110	0.449*	-0.092
		BC-220	0.628**	-0.154
	Split	Split-110	0.640**	-0.138
		Split-220	0.697**	-0.245

Note: NO_3^--N - Nitrate and NH_4^+-N- Ammonium, *, **, Significant at $p \leq 0.05$and 0.01, respectively.

Table A4. Spearman correlations of N_2O emissions with N cycling genes as affected by rate and timing of N application in soil under irrigated canola in 2015 and 2016

Year	Timing	Rate	AOA *amoA*	AOB *amoA*	*nirS*	*nirK*	*nosZ*
2015	Control	Control	0.371	-0.408	0.553**	-0.354	0.335
	Broadcast	BC-110	0.357	-0.170	0.657**	-0.031	0.413
		BC-220	0.410	-0.321	0.297	-0.456*	0.472*
	Split	Split-110	0.594**	-0.338	0.226	-0.692**	0.532**
		Split-220	0.329	-0.269	0.044	-0.616**	0.333
2016	Control	Control	-0.002	0.203	-0.280	0.184	-0.005
	Broadcast	BC-110	0.057	0.211	0.078	0.240	0.147
		BC-220	0.119	0.435	0.122	0.495*	0.306
	Split	Split-110	-0.080	0.612**	0.134	0.515*	-0.117
		Split-220	-0.167	0.474*	0.390	0.709**	-0.026

Note: AOA-Ammonia Oxidizing Archaea and AOB – Ammonia Oxidizing Bacteria.
*, **, Significant at $p \leq 0.05$ and 0.01 respectively.

Table A5. Spearman correlations of N_2O emissions with GWC and enzyme activity as affected by management events in soil under irrigated canola in 2015 and 2016

Year	Events	GWC	DEA	Urease	Arylamidase	P. nitrification
2015	Pre-Seeding	0.532*	0.173	-0.179	0.000	-0.138
	Post-Seeding	0.448*	----	-0.507*	0.336	0.254
	Pre-Irrigation	-0.195	0.165	-0.340	-0.181	0.469*
	Post-Irrigation	-0.071	----	----	----	0.271
	Pre-Bolt Fertilization	-0.375	-0.149	-0.247	-0.174	0.571**
	Post -Bolt Fertilization	0.275	-0.181	-0.070	-0.047	0.722**
2016	Pre-Seeding	----	----	----	----	----
	Post-Seeding	0.355	----	0.062	-0.042	0.271
	Pre-Irrigation	0.528*	0.423	-0.151	-0.094	0.533*
	Post-Irrigation	0.566**	----	-0.011	-0.065	0.460*
	Pre-Bolt Fertilization	-0.091	-0.256	-0.023	-0.141	0.211
	Post -Bolt Fertilization	0.233	0.411	0.105	0.306	0.525*

Note: GWC-Gravvimetric water content, DEA – Denitrifying enzyme activity, P – Potential and ---- missing. data, *, **, Significant at $p \leq 0.05$ and 0.01 respectively.

Table A6. Spearman correlations of N_2O emissions with NO_3^--N and NH_4^+-N as affected by rate and timing of N application in soil under irrigated canola in 2016

Year	Events	NO_3^--N	NH_4^+-N
2016	Post-Seeding	0.188	0.281
	Pre-Irrigation	0.640**	0.733**
	Post-Irrigation	0.667**	0.401
	Pre-Bolt Fertilization	0.027	-0.193
	Post -Bolt Fertilization	0.805**	-0.235

Note: NO_3^--N - Nitrate and NH_4^+-N- Ammonium, *, **, Significant at $p \leq 0.05$ and 0.01 respectively,

Table A7. Spearman correlations of N_2O emissions with N cycling genes as affected by management events in soil under irrigated canola in 2015 and 2016

Year	Events	AOA *amoA*	AOB† *amoA*	*nirS*	*nirK*	*nosZ*
2015	Pre-Seeding	-0.093	-0.150	0.230	-0.361	-0.126
	Post-Seeding	0.003	0.025	0.609**	-0.101	0.221
	Pre-Irrigation	-0.245	-0.021	0.052	-0.087	0.039
	Post-Irrigation	0.301	0.438	0.169	0.478*	0.332
	Pre-Bolt Fertilization	-0.307	0.322	-0.140	-0.076	-0.169
	Post -Bolt Fertilization	-0.327	0.074	-0.072	-0.219	-0.379
2016	Pre-Seeding	----	----	----	----	----
	Post-Seeding	-0.450*	0.143	0.067	-0.330	-0.210
	Pre-Irrigation	-0.039	0.513*	0.234	0.219	0.054
	Post-Irrigation	0.066	0.367	0.491*	0.432	0.537*
	Pre-Bolt Fertilization	-0.259	-0.101	-0.074	-0.047	0.030
	Post -Bolt Fertilization	0.271	0.057	-0.269	0.393	0.288

Note: AOA-Ammonia Oxidizing Archaea, AOB – Ammonia Oxidizing Bacteria and ---- missing data.
*, **, Significant at $p \leq 0.05$ and 0.01 respectively.

APPENDIX B

Correlation data for residue decomposition study

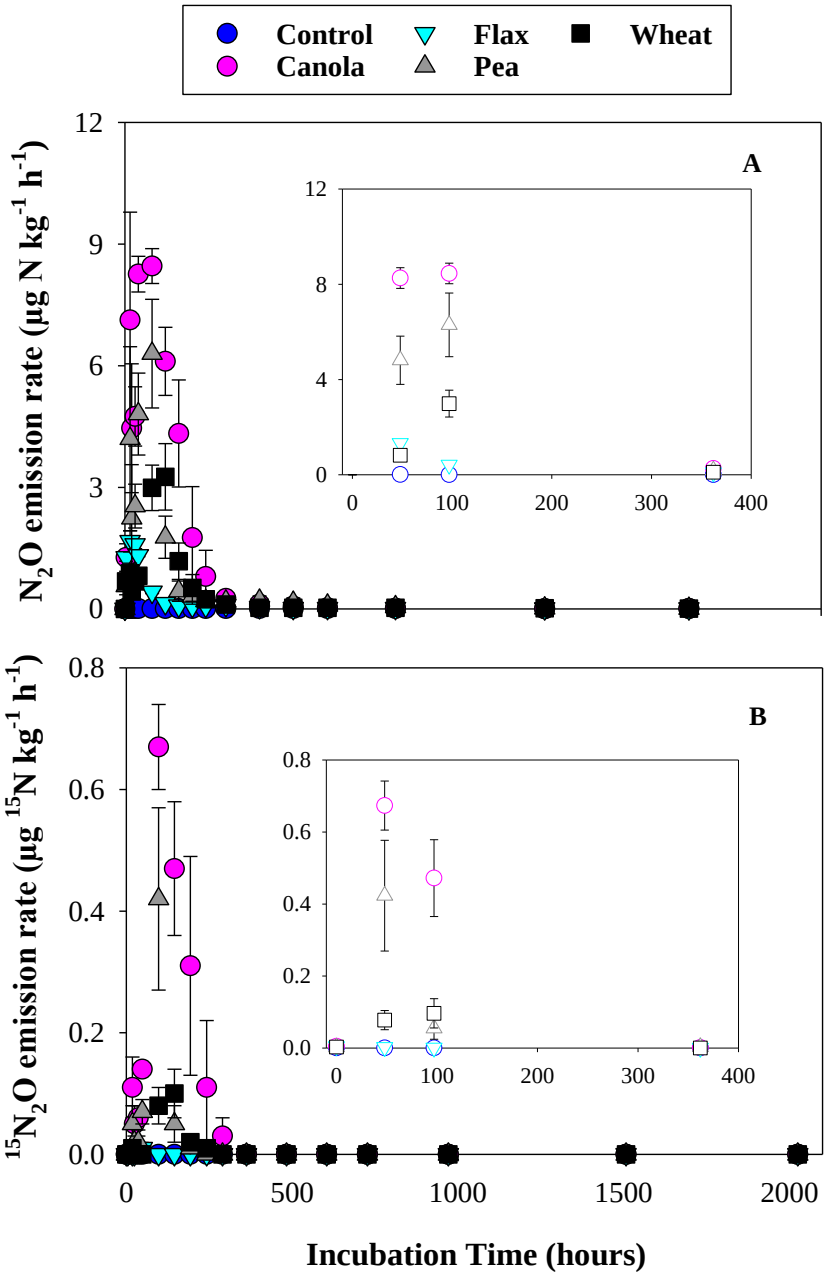


Fig. B1. Effect of canola, flax, pea and wheat residue addition on (A) N_2O emission rate and (B) $^{15}N_2O$ emission rate (insert shows emission rate at destructive sampling). Error bars indicate standard error of the mean (n=4).

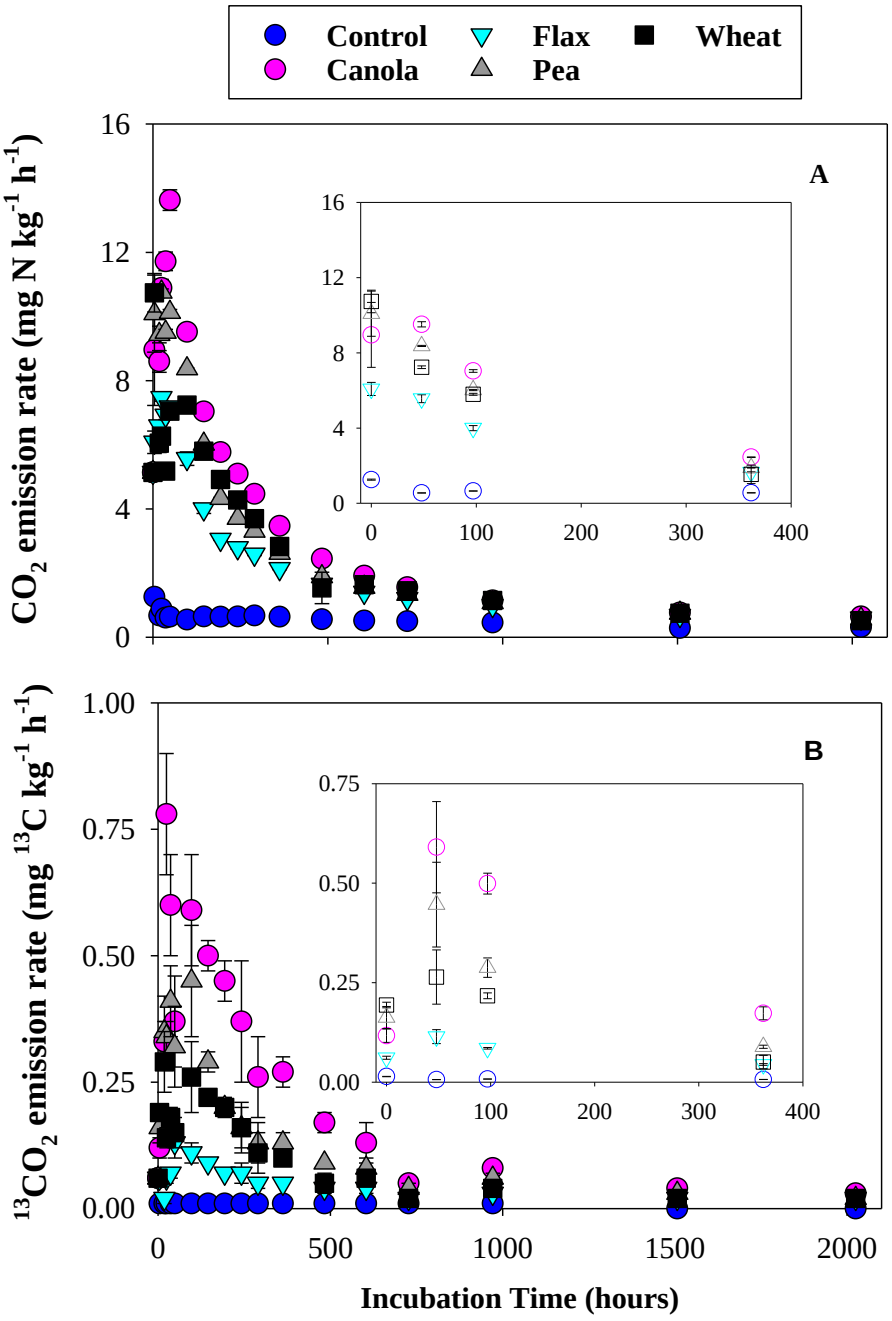


Fig. B2. Effect of canola, flax, pea and wheat residue addition on (A) CO_2 emission rate and (B) $^{13}CO_2$ emission rate (insert shows emission rate at destructive sampling). Error bars indicate standard error of the mean (n=4).

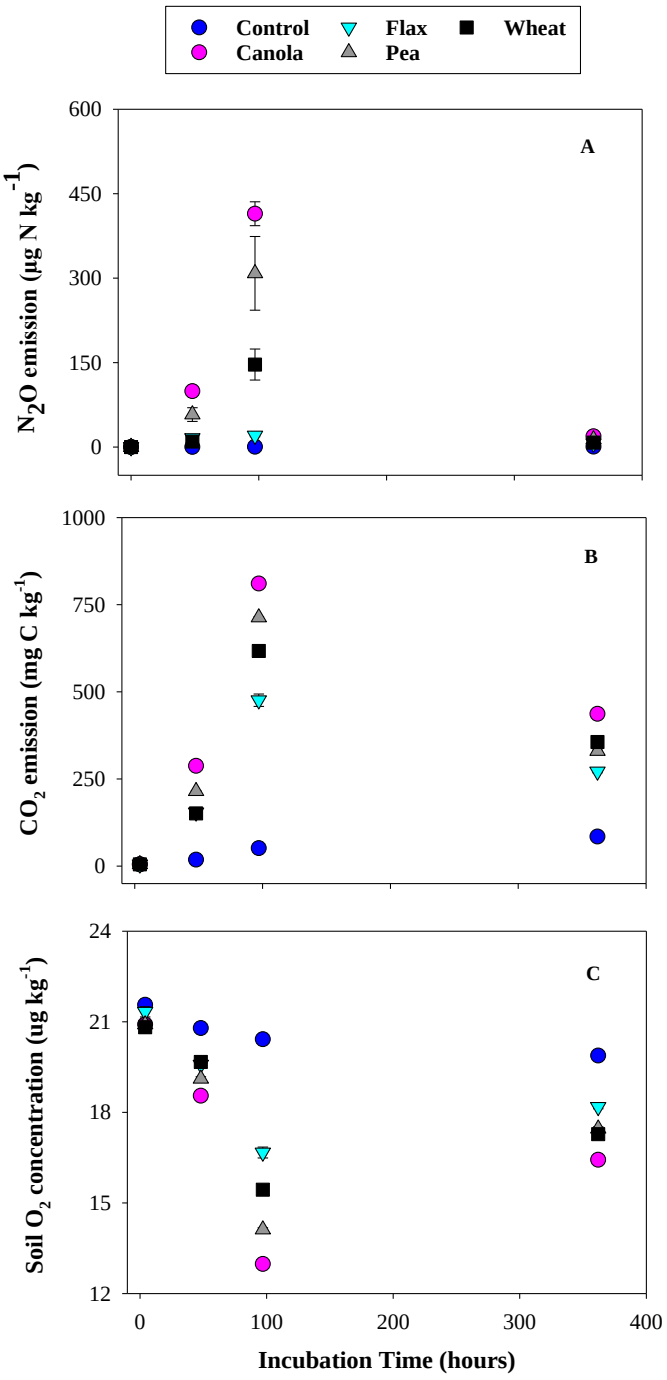


Fig. B3. Effect of canola, flax, pea and wheat residue addition on (A) N_2O emission (B) CO_2 emission and (C) soil O_2 concentration at 0, 48, 97 and 362 h sampling. Error bars indicate standard error of the mean (n=4).

Table B1. Effect of labelled residues of canola, flax, pea and wheat application on abundance of N-cycling functional genes in incubated soils at four sampling times (n = 4).

Time (h)	Crop residue	AOA	AOB	*nirS*	*nirK*	*nosZ* I	*nosZ* II	*nirS* : *nirK*	*nosZ* I : *nosZ* II	(*nirS*+ *nirK*) : *nosZ* I	(*nirS*+ *nirK*) : *nosZ* II
		……log gene copies g soil $^{-1}$……						….....….....Ratio….....			
0	Control	9.11	7.89	6.66	7.59 b	7.43 b	8.90	0.88	0.83 b	1.92	1.60
	Canola	9.18	7.92	6.72	7.59 b	7.51 ab	8.92	0.89	0.84 ab	1.91	1.60
	Flax	9.24	7.94	6.76	7.80 a	7.65 a	8.93	0.87	0.86 a	1.90	1.63
	Pea	9.07	7.73	6.78	7.65 ab	7.46 ab	8.97	0.89	0.83 b	1.93	1.61
	Wheat	9.08	7.77	6.69	7.55 ab	7.45 ab	8.95	0.89	0.83 b	1.91	1.59
48	Control	9.41	8.25	7.23 b†	8.28 b	7.61 c	8.32	0.87	0.91 b	2.04 a	1.87
	Canola	9.75	8.29	7.56 a	8.72 a	8.33 a	8.61	0.87	0.97 a	1.95 b	1.89
	Flax	9.39	8.17	7.42 a	8.59 a	7.82 b	8.47	0.86	0.92 ab	2.05 a	1.89
	Pea	9.51	8.12	7.47 a	8.49 a	7.88 b	8.42	0.88	0.94 ab	2.03 a	1.89
	Wheat	9.54	8.32	7.35 ab	8.66 a	8.03 b	8.48	0.85	0.95 a	1.99 ab	1.89
97	Control	9.54	8.05	8.21 b	7.38 b	7.70 b	8.36	1.11	0.92	2.03 a	1.87
	Canola	9.42	8.09	8.46 ab	7.46 ab	7.98 a	8.37	1.14	0.95	1.99 b	1.90
	Flax	9.67	8.10	8.50 a	7.67 a	7.92 a	8.55	1.11	0.93	2.04 a	1.89
	Pea	9.61	8.05	8.47 a	7.68 a	7.97 a	8.54	1.10	0.93	2.03 a	1.89
	Wheat	9.58	8.02	8.47 ab	7.77 a	7.92 a	8.59	1.09	0.92	2.05 a	1.89
362	Control	9.43	8.16	7.14 b	7.95 b	8.18 b	8.76 b	0.90	0.93 b	1.85	1.72
	Canola	9.51	8.15	7.46 a	8.36 a	8.62 a	9.07 a	0.89	0.95 a	1.83	1.74
	Flax	9.35	8.08	7.35 a	8.01 ab	8.38 a	8.84 ab	0.92	0.95 ab	1.83	1.74
	Pea	9.43	7.99	7.43 a	8.17 a	8.37 ab	9.02 a	0.91	0.93 ab	1.86	1.73
	Wheat	9.37	7.99	7.34 ab	8.15 ab	8.41 a	8.93 ab	0.90	0.94 ab	1.84	1.73

† Different letters within the same column indicate a significant difference between means.

Table B2. Effect of labelled residues of canola, flax, pea and wheat application on microbial biomass in incubated soil at four different sampling time (n=4).

Time (h)	Crop residue	Total PLFA	Bacteria	Gram +	Gram -	A.Bacteria	Fungi	AMF	Stress 1	Stress 2
		nmol g^{-1} of dry soil								
0	Control	45.57	27.40	10.99	11.89 c	4.52	0.63 c	2.02	0.38 a	0.33 a
	Canola	66.19	38.21	13.73	19.06 a	5.42	2.85 a	2.23	0.31 b	0.21 b
	Flax	52.29	29.37	11.35	13.20 bc	4.83	2.26 ab	1.95	0.35 a	0.29 a
	Pea	62.77	33.20	12.11	16.27 a	4.82	2.58 ab	2.04	0.33 b	0.22 b
	Wheat	58.11	33.82	12.62	16.18 ab	5.02	1.72 bc	2.25	0.34 ab	0.26 ab
48	Control	58.52 c†	35.13 c	14.56 c	14.14 d	6.43	0.72 c	2.40 b	0.42 a	0.41 a
	Canola	104.79 a	64.21 a	24.67 a	32.10 a	7.43	4.10 a	2.91 a	0.26 e	0.17 c
	Flax	85.48 b	51.84 b	21.55 b	23.07 c	7.22	2.62 b	2.57 b	0.34 b	0.24 b
	Pea	99.96 a	60.87 a	24.35 a	28.86 b	7.67	3.60 a	2.88 a	0.28 d	0.20 b
	Wheat	89.21 ab	52.03 b	20.59 b	24.32 c	7.12	2.27 b	2.88 a	0.31 c	0.21 b
97	Control	57.02 b	34.32 b	13.71 b	14.40 b	6.21 b	0.73 c	2.42 b	0.42	0.38 a
	Canola	79.49 a	46.78 a	18.02 a	21.70 a	7.07 ab	2.44 a	3.39 a	0.42	0.33 b
	Flax	78.85 a	44.66 a	17.29 ab	20.27 a	7.10 ab	2.09 b	3.20 ab	0.44	0.34 ab
	Pea	80.20 a	47.25 a	18.52 a	21.36 a	7.37 a	1.94 b	3.45 a	0.42	0.32 b
	Wheat	69.77 a	40.70 ab	16.16 ab	18.10 ab	6.49 ab	1.31 bc	3.00 ab	0.42	0.36 ab
362	Control	56.80 c	34.39 c	14.41 c	13.92 c	6.06 c	0.62 d	2.48 b	0.40 a	0.38 a
	Canola	101.68 a	61.57 a	23.15 a	30.54 a	7.88 a	3.37 ab	3.41 a	0.30 d	0.20 c
	Flax	84.67 b	50.91 b	20.54 b	23.11 b	7.02 b	2.64 b	2.96 b	0.37 b	0.24 b
	Pea	93.17 ab	55.27 ab	21.51 ab	26.46 ab	7.30 ab	3.47 a	3.12 ab	0.31 cd	0.23 b
	Wheat	89.57 b	53.32 b	20.38 b	25.37 b	7.57 a	2.30 c	3.28 a	0.32 c	0.23 b

† Different letters within the same column indicate a significant difference between means.

Table B3. Pearson correlations of biogeochemical properties of incubated soil with total microbial abundance as affected by labelled canola, flax, pea and wheat residue application.

Abundance	Treatments	DOC	DON†	DOC/DON	GWC	NO_3^-	NH_4^+
Total PLFA	Control	-0.124	0.692**	-0.806**	-0.776**	-0.499*	0.767**
	Canola	-0.493	0.266	-0.715**	-0.672**	-0.642**	0.283
	Flax	-0.676**	0.484	-0.847**	-0.954**	-0.620*	-0.524*
	Pea	-0.318	0.197	-0.417	-0.688**	-0.536*	0.289
	Wheat	-0.454	0.383	-0.734**	-0.647**	-0.655**	0.169

Note: DOC-Dissolved organic carbon, DON – Dissolved organic nitrogen , GWC – Gravimetric water content, NO_3^- - Nitrate and NH_4^+ – Ammonium, *, **, *** Significant at $p \leq 0.05$, 0.01, and 0.001, respectively.

Table B4. Pearson correlations of biogeochemical properties of incubated soil with fungal abundance as affected by labelled canola, flax, pea and wheat residue application.

Abundance	Treatments	DOC	DON	DOC/DON	GWC	NO_3^-	NH_4^+
Fungi	Control	0.083	0.217	-0.150	-0.166	-0.052	0.272
	Canola	0.004	0.704**	-0.678**	-0.178	-0.082	0.287
	Flax	-0.088	0.177	-0.184	-0.371	0.039	-0.457
	Pea	0.157	0.226	0.022	-0.224	-0.049	-0.269
	Wheat	-0.089	0.507*	-0.664**	-0.158	-0.257	-0.186

Note: DOC-Dissolved organic carbon, DON – Dissolved organic nitrogen , GWC – Gravimetric water content, NO_3^- - Nitrate and NH_4^+ – Ammonium, *, **, *** Significant at $p \leq 0.05$, 0.01, and 0.001, respectively.

Table B5. Pearson correlations of biogeochemical properties of incubated soil with bacterial abundance as affected by labelled canola, flax, pea and wheat residue application.

Abundance	Treatments	DOC	DON	DOC/DON	GWC	NO_3^-	NH_4^+
Bacteria	Control	-0.155	0.681**	-0.813**	-0.786**	-0.483	0.760**
	Canola	-0.512*	0.278	-0.744**	-0.678**	-0.646**	0.291
	Flax	-0.608*	0.499*	-0.805**	-0.935**	-0.531*	-0.551*
	Pea	-0.417	0.215	-0.528*	-0.750**	-0.615*	0.357
	Wheat	-0.491	0.344	-0.723**	-0.651**	-0.671**	0.137

Note: DOC-Dissolved organic carbon, DON – Dissolved organic nitrogen , GWC – Gravimetric water content, NO_3^- - Nitrate and NH_4^+ – Ammonium, *, **, *** Significant at $p \leq 0.05$, 0.01, and 0.001, respectively.

Table B6. Pearson correlations of biogeochemical properties of incubated soil with gram +ve bacteria abundance as affected by labelled canola, flax, pea and wheat residue application.

Abundance	Treatments	DOC	DON	DOC/DON	GWC	NO_3^-	NH_4^+
Gram +ve	Control	-0.161	0.703**	-0.839**	-0.804**	-0.501*	0.728**
	Canola	-0.555*	0.261	-0.764**	-0.717**	-0.681**	0.320
	Flax	-0.550*	0.552*	-0.800**	-0.907**	-0.461	-0.541*
	Pea	-0.428	0.239	-0.562*	-0.764**	-0.625**	0.396
	Wheat	-0.514*	0.365	-0.750**	-0.709**	-0.719**	0.231

Note: DOC-Dissolved organic carbon, DON – Dissolved organic nitrogen , GWC – Gravimetric water content, NO_3^- - Nitrate and NH_4^+ – Ammonium, *, **, *** Significant at $p \leq 0.05$, 0.01, and 0.001, respectively.

Table B7. Pearson correlations of biogeochemical properties of incubated soil with gram -ve bacteria abundance as affected by labelled canola, flax, pea and wheat residue application.

Abundance	Treatments	DOC	DON	DOC/DON	GWC	NO_3	NH_4^+
Gram -ve	Control	-0.176	0.527*	-0.665**	-0.654**	-0.420	0.687**
	Canola	-0.422	0.358	-0.740**	-0.608*	-0.576*	0.282
	Flax	-0.611*	0.465	-0.786**	-0.941**	-0.557*	-0.571*
	Pea	-0.358	0.212	-0.461	-0.700**	-0.552*	0.269
	Wheat	-0.427	0.363	-0.709**	-0.562*	-0.607*	0.040

Note: DOC-Dissolved organic carbon, DON – Dissolved organic nitrogen , GWC – Gravimetric water content, NO_3^- - Nitrate and NH_4^+ – Ammonium, *, **, *** Significant at $p \leq 0.05$, 0.01, and 0.001, respectively.

Table B8. Pearson correlations of biogeochemical properties of incubated soil with gram -ve bacteria abundance as affected by labelled canola, flax, pea and wheat residue application.

Abundance	Treatments	DOC	DON	DOC/DON	GWC	NO_3	NH_4^+
A†. Bacteria	Control	-0.079	0.805**	-0.898**	-0.863**	-0.482	0.836**
	Canola	-0.737**	-0.136	-0.558*	-0.785**	-0.774**	0.167
	Flax	-0.722**	0.470	-0.873**	-0.918**	-0.608*	-0.392
	Pea	-0.570*	0.106	-0.605*	-0.813**	-0.757**	0.528*
	Wheat	-0.628**	0.156	-0.610*	-0.754**	-0.705**	0.219

Note: DOC-Dissolved organic carbon, DON – Dissolved organic nitrogen , GWC – Gravimetric water content, NO_3^- - Nitrate and NH_4^+ – Ammonium, *, **, *** Significant at $p \leq 0.05$, 0.01, and 0.001, respectively.